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(54) Title: EPITHELIAL MEMBRANE PROTEIN-1

(57) Abstract

Epithelial membrane protein-1 (EMP-1) was identified as having a high degree of homology with peripheral myelin protein 22 (PMP22) and was expressed predominantly in the digestive tract and the nervous system. EMP-1 and PMP22 define a novel family of transmembrane proteins which are involved in the regulation of cell growth and/or differentiation. Included in the invention are EMP-1 nucleic acids, and expression vectors and host cells for the recombinant production of EMP-1. Nucleic acids are used to detect EMP-1 mutation in biological samples and to treat EMP-1 related conditions by anti-sense or gene therapy. Host cells displaying EMP-1 are used to screen for EMP-1 ligands and for receptor agonists and antagonists.

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EPITHELIAL MEMBRANE PROTEIN-1

Field of the Invention

The present invention relates to transmembrane proteins which act in signal transduction to modulate the growth and/or differentiation of target cells. More particularly, the invention relates to epithelial membrane protein-1 (EMP-1), recombinant production of EMP-1, and methods and reagents for modulating the activity and expression of EMP-1.

Background of the Invention

An understanding of the mechanisms involved in cellular growth and differentiation promises to be useful in elucidating the causes of and providing treatments for human disease. One area of research has focused on identification of cell surface receptors which act in signal transduction. Cell surface receptors are transmembrane proteins which transfer a stimulus from the outside environment of the cell to intracellular molecules which in turn impart a change in cell physiology. This change in physiology is typically one involving the growth or differentiation state of the cell. A number of receptor families consisting of structurally and functionally related members have been identified (see review by Van der Geer et al. Ann. Rev. Cell Biol. 10, 251-337 (1994).

Generally cell surface receptors transduce a signal to the cell interior upon interaction with a ligand. The ligand, usually a polypeptide, typically induces a conformational change in the receptor which triggers the signalling process. This process is

referred to as receptor activation and may involve events such as receptor oligomerization (either with the same or with different receptors) and receptor tyrosine phosphorylation. While polypeptide ligands studied so far will preferentially interact with a single receptor a given ligand may also bind to and activate one or more other members within a receptor family.

Techniques for rapidly isolating and sequencing DNA have greatly accelerated the search for receptors and their cognate ligands. In particular, random sequencing of cDNA libraries to generate expressed sequence tags (ESTs) along with computational methods which compare newly obtained ESTs with nucleic acid sequences in sequence databases now permit the rapid identification of sequences having high degrees of homology. It is now possible to rapidly assign a new sequence (full or partial) to a family of related sequences and, in doing so, to deduce one or more likely functions of the encoded proteins. In addition, knowledge of highly conserved stretches of nucleic acid or amino acid sequence in families of related sequences allows one to design oligonucleotide probes for screening CDNA libraries for related molecules.

The identification of related members of a single family of receptors or ligands provides valuable insight into the structure and function of that family. Related members of a family lead to an understanding of the role of receptor family members, their cognate ligands, and accessory proteins in cellular physiology. This information aids in the development of therapies based upon the stimulation or blocking of receptor and/or ligand functions.

It is an object of the invention to identify sequences which encode receptors or their cognate ligands. It is a further object to understand structure/function relationships of new receptor or ligand sequences to existing sequences. A nucleic acid sequence has been found that is structurally related to peripheral myelin protein-22 (PMP22) and with PMP22 defines a new family of receptors.

PMP22, or peripheral myelin protein 22, is a transmembrane protein that has been previously identified as an expression product primarily in Schwann cells (Welcher et al. Proc. Natl. Acad. Sci. USA 88, 7195-7199 (1991); Spreyer et al. EMBJ. 10, 3661-3668 (1991)). Point mutations or gene arrangements in PMP22 or abnormal expression of the corresponding gene lead to motor and sensory neurophathies of the peripheral nervous system (PNS) (Suter et al., TINS 16, 50-56 (1993)). Human diseases associated with alterations in the PMP22 gene include a variety of neuropathies such as Charcot-Marie-Tooth disease type 1A and Dejerine-Sottas syndrome. (Matsunami et al. Nature Genet. 1, 176-179 (1992); Patel et al. Nature Genet. 1, 159-165 (1992); Roa et al. Nature Genet. 5, 269-272 (1993)). In addition, PMP22 was observed to be upregulated under growth arrest conditions in cell culture, suggesting a role for PMP22 in cell proliferation. However, no known PMP22 mutants show any phenotype other than that observed in the PNS, suggesting that related molecules may compensate for the lack of functional PMP22 in non-neural tissues. Further, there has been no significant homology observed between the amino acid sequence of PMP22 and any other known protein. A

description of PMP22 and its involvement in PNS disorders has been described in PCT Application No. 92/21694 which is hereby incorporated by reference

The identification of PMP22-related molecules provides potential therapies based upon regulation of the growth and/or differentation of tissues which bear PMP22 related molecules on their surface and which respond to external stimuli recognized by PMP22-related molecules. Identification of PMP22-related molecules allows one to design compounds for the control of cell growth and/or differentiation.

Summary of the Invention

The present invention provides for epithelial membrane protein-1 (EMP-1) which has significant sequence homology to PMP-22 and along with PMP-22 defines a new family of receptors. EMP-1 is involved in the control of growth and/or differentiation of the various tissues in which it is expressed. In particular, EMP-1 may function in the nervous system, lung and in the epithelial cells lining the gastrointestinal tract.

Isolated nucleic acid molecules encoding EMP-1 are provided. EMP-1 is preferably mammalian in origin and may be mouse, rat or human. The nucleic acids may also be variants which are naturally occurring or constructed by *in vitro* mutagenesis of the EMP-1 nucleic acid sequence. DNA molecules comprising an expression system capable of expressing EMP-1 and host cells modified with said DNA molecules are also encompassed. Preferably, the modified host cells will display EMP-1 at the cell surface. The nucleic acids may also be used

in gene therapy or anti-sense therapy for treatment of disorders related to EMP-1 as well as disorders related to EMP-1 expressing cells.

A method for producing host cells displaying EMP-1 is also provided by the invention. Said host cells may be used to screen for agonists or antagonists of EMP-1 and for proteins which interact with EMP-1.

Description of the Figures

Figure 1. cDNA and predicted amino acid sequence of rat EMP-1. The rat EMP-1 cDNA clone contains an open reading frame of 480 base pairs starting with an ATG codon at nucleotide 132 and terminating with a TAA stop codon at position 612. A potential cleavable signal peptide spans amino acid residues 1 to 16. A single motif for putative N-linked glycosylation is present between the first and second hydrophobic domains at asparagine 43.

Figure 2. cDNA and predicted amino acid sequence of mouse EMP-1. The mouse cDNA clone contains as open reading frame of 480 base pairs starting with an ATG codon at nucleotide 183 and terminating with a TAA codon at nucleotide 663. A potential cleavable signal peptide spans amino acid residues 1 to 16. The potential N-linked glycosylation site is conserved from the rat sequence.

Figure 3. cDNA and predicted amino acid sequence of human EMP-1. The human cDNA clone contains an open reading frame of 471 base pairs starting with an ATG codon at nucleotide 8 and terminating with a TAA stop codon at position 479. The potential signal

peptide spans residues 1 to 16. The potential N-liked glycosylation site is conserved from the rat and mouse sequences.

Figure 4. Predicted EMP-1 structure and amino acid sequence comparison of PMP22/EMP family members. **A.** Comparison of predicted amino acid sequences of rat EMP-1 with rat, mouse and human PMP22. **B.** Hypothetical topology of EMP-1 in a lipid bilayer based on computer-assisted hydrophobicity plots and secondary structure predictions. Residues identical to rat PMP22 are filled. Most of the point mutations in PMP22 known to result in hereditary peripheral neuropathies (diamonds) are conserved at the corresponding positions in EMP-1. The Y-shaped symbol indicates a potential N-linked carbohydrate chain. **C.** Amino acid comparison of the three known PMP22/EMP/MP20 family members. The residue conserved between MP20 and PMP22 or EMP-1 as shown in reversed type.

Figure 5. *In vitro* transcription and translation of EMP-1 and PMP22 cDNAs. The [^{35}S]methionine metabolically labelled proteins were separated by reducing 15% SDS PAGE. pcDNA-1 was used as a control and no specific proteins can be detected. The EMP-1 cDNA generated an 18 kDa protein which clusters (EMP-1). Transcription and translation of the EMP-1 cDNA in the presence of CMM results in a reduced rate of migration of the protein (EMP-1 + CMM). This reduced migration is reversed by deglycosylation with N-Glycosidase F (EMP-1 deglycosylated). The PMP22 cDNA generates an 18 kDa protein whose apparent molecular weight increases by 4-6 kDa when the reaction is performed in the presence of CMM (PMP22 + CMM).

Treatment with N-Glycosidase F reduced the molecular weight back to 18 kDa (PMP22 deglycosylated). Neither EMP-1 nor PMP22 are substrates for signal peptidase *in vitro* as indicated by the identical migration rate of the unglycosylated (EMP-1, PMP22) and deglycosylated translation products (EMP-1 + CMM, PMP22 + CMM). Prolactin (**a**) is a substrate for signal peptidase, approximately 50% of the protein is processed when translated in the presence of CMM (**c**) (Promega Technical manual, Promega Biotech, Madison, WI). α-factor was used as a control for N-linked glycosylation and was completely modified in the reactions (**b**) (Promega Technical manual),.

Figure 6. Tissue distribution of EMP-1 and PMP22 mRNAs in the rat. Northern blot analysis with a radiolabelled EMP-1 probe shows high expression of 2.8 kb transcripts in the caecum, colon, rectum, fundus and ileum (**a**). Lower levels of expression are observed in the duodenum and jejunum of the small intestine and the corpus and pylorus of the stomach (**a**). Additional transcripts of 1.7 kb are found in the fundus, ileum, caecum and colon (**a**). In extraintestinal tissues, EMP-1 mRNA levels are high in the skin, whereas in the brain and lung, expression is comparable to the duodenum (**b**; panels **a** and **c** are 20 hour exposures, and **b** and **d** 48 hours exposures of the same blot). PMP22 mRNA is also highly expressed in the intestine (**c**); its 1.8 kb transcript is most prominent in the rectum and caecum where expression is comparable to that of PMP22 in the lung (**c**, **d**). Ten μg of total RNA was loaded per lane and equal loading was verified by ethidium bromide staining.

Figure 7. Regulation of EMP-1 and PMP22 mRNA expression by sciatic nerve injury and in cultured cells. **A.** Northern blot analysis of EMP-1 mRNA reveals an increased expression in the degenerating distal part of the injured sciatic nerve (4 days after nerve cut) compared to normal control nerve. PMP22 expression is considerably higher than that of EMP-1 in the normal nerve (1 hour exposure using the PMP22 probe compared to 36 hours for the EMP-1 probe). In contrast to EMP-1, PMP22 mRNA is dramatically reduced in the distal nerve after injury. **B.** Cultured, mitogen-expanded primary rat Schwann cells (pSC) and D6P2T Schwann cells display reduced EMP-1 expression following forskolin treatment. In contrast, PMP22 mRNA expression is increased under the same conditions. **C.** Serum starvation-induced growth arrest of NIH3T3 cells results in reduced EMP-1 mRNA expression and an increase in PMP22 expression relative to exponentially growing cells. Northern blot analyses were performed on the same blot (10 μg total RNA per sample) which was stripped between hybridizations.

Figure 8. Expression of EMP-1 protein in the rat intestine. **A.** Two rabbit anti-EMP-1 peptide antisera raised against each of the putative extracellular loops 1 and 2 of EMP-1 recognize a 25 kDa protein in the corpus gastricum (50 μg protein lysate analysed by 12% SDS-PAGE and Western blotting). The immune reactivity of the anti-loop1 antiserum was blocked by preincubation with 250 μg/ml of the immunogen but not by the loop2 peptide. **B.** Strong expression of EMP-1 protein is found in the stomach and large intestine, lower levels are present in the lung. Detection of a signal in the small intestine requires prolonged reaction time of the enzymatic detection system.

Figure 9. Detection of transiently expressed EMP-1 in COS cells. COS cells were transiently transfected with an EMP-1 expression construct and subsequently analysed by immunofluorescence using the anti-loop2 antiserum and a FITC-labelled goat anti-rabbit Ig. Identical results were obtained using the anti-loop1 antiserum. The scale bar represents 20 µm.

Figure 10. Immunofluorescent localization of EMP-1 protein expression in the corpus gastricum. **a)** Schematic view of the gastric mucosa showing the proliferative zone in the neck/isthmus region of the gastric gland and the migration of the differentiating epithelial cells towards the gastric pit. **b)** Low magnification view of the gastric mucosa labelled with the polyclonal anti-EMP-1 loop2 antiserum and detected with a Texas Red-labelled donkey anti-rabbit antibody. Intense immunoreactivity can be detected in the epithelial cells of the outer mucosa. No immunoreactive cells can be found towards the base of the gastric pit or in the sub-mucosal muscle layer (sm). The intense labelling of the isolated cells at the base of the gastric mucosa is not specific as it is also present in control sections incubated with preimmune serum (not shown). **c)** Higher magnification of the labelled epithelial cells in the pit region. The migrating, differentiating epithelial cells in the isthmus express high levels of EMP-1 protein. **d)** Transmitted light view of the region shown in panel c. **e)** Cross section through the gastric pit shows intense plasmamembrane-associated labelling of the epithelial cells but no labelling of the mesenchyme. No staining is seen in transverse sections across the base of the gastric gland (not

shown). **f**) Transmitted light view of the section shown in panel e. The scale bars shown are 100 µm for **b** and 60 µm for panels **c** to **f**. sm- sub mucosal muscle layer.

Detailed Description of the Invention

cDNA libraries can be sequenced at random to generate rapidly many partial sequences referred to as expressed sequence tags (ESTs). Two ESTs obtained from a mouse and rat cDNA library were observed to have significant homology with the corresponding PMP22 DNA sequences. Further analysis revealed the full length coding sequences for the protein, termed epithelial membrane protein-1 (EMP-1). These sequences were used as probes to isolate the corresponding human sequence by screening a human lung cDNA library.

Computer-assisted analysis predicted the following properties for rat, mouse and human EMP-1. The proteins are highly hydrophobic, and likely contain four transmembrane regions. The transmembrane domains in rat EMP-1 are predicted to span amino acid residues 1-28, 64-89, 95-117 and 134-157. Two predicted extracellular domains encompass the amino acid residues 29-63 and 113-133. These proteins are therefore integral membrane proteins and are likely to be either a receptor or a channel. The only sequence that showed homology to these proteins in the GenBank database is PMP22. EMP-1 and PMP22 show 40% amino acid identity and a very striking conservation of predicted structure. Comparison across EMP-1 species showed that the mouse and human protein had 78% identity. Based on these comparisons, PMP22, EMP-1 and the previously cloned lens

membrane protein 20 (MP20) define a new family of proteins.

The high degree of identity at the amino acid level suggests that EMP-1 and PMP22 may serve similar functions. Close examination of the amino acid sequences of these proteins reveals that the hydrophobic regions, in particular the first two transmembrane domains, are highly conserved suggesting that they are of particular functional importance. This hypothesis is further supported by the finding that the hydrophobic domains are the most strongly conserved regions between PMP22 species homologues. Interestingly, the amino acid residues in PMP22 that are sites of mutation in hereditary peripheral neuropathies are located within putative transmembrane domains and the majority of these mutated amino acid residues are also conserved at the corresponding positions of EMP-1 and MP20.

A conserved feature within the putative extracellular domains of EMP-1 and PMP22 is the consensus sequence for an N-linked glycosylation. This glycosylation site in PMP22 carries a modified carbohydrate chain containing the L2/HNK-1 epitope, a structure which has been implicated in cell-cell recognition and adhesion processes (for recent review see Schachner et al., TINS 18, 183-191 (1995)). Although the presence and nature of carbohydrate moieties linked to EMP-1 remains to be determined, an N-linked glycosylation in the identical position of EMP-1 may be involved in cell recognition processes in the epithelium of the intestine.

Expression of EMP-1 and PMP22

The tissue distribution of mRNA for rat EMP-1 and rat PMP22 is shown in Figure 6. EMP-1 mRNA is

highly expressed in the adult digestive system including cecum, colon, ileum, jejunum, colonic crypts, duodenum, and stomach. Lower level expression is also seen in most other tissues including the skin, heart, brain, thymus, lung, and kidney. The expression in the digestive system is similar to that seen for PMP22. Although EMP-1 mRNA is expressed in peripheral nerve, it is expressed at much lower levels than PMP22. EMP-1 mRNA is expressed in a broader range of tissues than is PMP22. EMP-1 mRNA was detected in most human adult tissues examined, including heart, brain, lung, muscle, kidney, colon, and small intestine.

Regulation of EMP-1 and PMP22 expression was studied in several different systems. In cut sciatic nerve, where Schwann cells are continually dividing, EMP-1 mRNA is increased, while PMP22 is decreased. In Schwann cells (both primary and cell lines), EMP-1 mRNA is downregulated following forskolin treatment, while PMP22 is increased. In fibroblasts, EMP-1 mRNA is associated with rapidly dividing cells, while PMP22 is associated with growth-arrested (non-dividing) cells. In aggregate, these three northern blots establish a correlation of EMP-1 mRNA expression with actively dividing cells, and suggest that the EMP-1 protein is needed for normal cell division.

Expression of recombinant rat EMP-1 protein in COS cells is shown in Figure 9. Recombinant human EMP-1 can be expressed as described in Example 6. EMP-1 protein is expressed in rat tissues that are making the mRNA, particularly those tissues in the digestive system. Within the digestive system, the EMP-1 protein is found to be expressed in the epithelial cells.

The striking homology of EMP-1 and PMP22 suggests possible functions for EMP-1. As indicated in

the Background section, mutations in PMP22 lead to several important human diseases, most of which seem to be caused by alterations in the growth state of expressing cells, that is cells divide or fail to divide at inappropriate times. It is likely that EMP-1 will be involved in controlling the proliferative state of cells as well. Because EMP-1 is expressed in a wide variety of tissues, it is anticipated that mutations in EMP-1 or changes in the expression of EMP-1 may be associated with abnormal proliferation/differentiation of a wide range of tissues.

The elevated levels of EMP-1 expression in the intestinal tract suggests a role for EMP-1 in the proliferation and differentiation of cells in this region. The gastrointestinal tract is characterized by a continual and rapid renewal of its epithelial surface which continues throughout the animals life. Pluripotent stem cells anchored in the isthmus/neck regions of the gastric gland give rise to progeny displaying increased proliferation and reduced potentiality which progress to terminally differentiated mature cells (Gordon et al., Curr. Opin. Cell Biol. 6, 795-803 (1994)). During this differentiation process, the cells are highly migratory, with proliferation, migration and differentiation all being tightly coupled. EMP-1 is found mainly in the proliferation and differentiation zones of the outer gastric gland as well as in the mature epithelial cells of the gastric pit region. In these cells, EMP-1 appears to be associated with the plasma membrane, with no clear distinction between the basal, apical and lateral aspects.

EMP-1 Nucleic acids

Isolated nucleic acid molecules encoding EMP-1 are encompassed by the invention. The molecules comprise sequences which encode EMP-1 as evidenced by generation of a recombinant protein of predicted size and post-translational modification similar to the endogenous protein. EMP-1 is preferably mammalian in origin and may be rat, mouse or human EMP-1 as shown in Figure 1, 2, and 3 (SEQ ID NOS: 1, 3 and 5) respectively. EMP-1 nucleic acids may also be variants of the sequences specifically disclosed, wherein a variant may be a naturally occurring alleleic variant or a substitution, deletion, or addition of one or more amino acids prepared by recombinant DNA techniques. The effects of mutation on EMP-1 biological activity may be predicted based upon corresponding mutation in related PMP22. PMP22 mutations leading to peripheral nervous system disorders are located in transmembrane domains which are highly conserved in EMP-1 (see Figure 4C). It is expected that mutations within these regions would also affect EMP-1 activity as well. Therefore, EMP-1 variants which retain biological activity are more likely to be found outside the conserved regions of the PMP/EMP family members shown in Figure 4C.

EMP-1 nucleic acids may be used as diagnostic reagents to study the structure of EMP-1 genes in biological samples. The molecules and reagents can be used to identify in a biological sample mutations or alterations in EMP-1 that may be predictive or diagnostic of pathological states, including cancer caused by abnormal proliferation of cells expressing EMP-1, and neurodegenerative disorders caused by an inability of cells expressing EMP-1 to maintain the normal physiology and differentiated state. The nucleic

acid sequences can also be used to design oligonucleotide primers to look for mutations in EMP-1 that may be responsible for various pathological states. The method comprises incubating a biological sample having an altered EMP-1 nucleic acid sequence with a full-length or partial EMP-1 sequence; isolating the EMP-1 nucleic acid in the sample; and identifying the mutation or alteration. Particular tissues which would be most affected by abnormalities in EMP-1 include the digestive system, the nervous system, and the lung.

EMP nucleic acids may also be used to identify related genes which are members of the PMP22/EMP-1 family. Nucleic acid probes can be made to conserved regions of EMP/PMP22 family members, such as the transmembrane domains, and used to screen cDNA or genomic libraries by hybridization or polymerase chain reaction (PCR) for related molecules.

Nucleic acids of the invention may also be used as reagents for gene therapy and anti-sense therapy to modify the expression of EMP-1 in selected tissues. EMP-1 expression may be increased by modifying tissue with an expression vector containing the EMP-1 coding region, wherein the vector produces EMP-1 in a tissue specific manner. Tissues that can be targeted for EMP-1 gene therapy include those associated with the digestive system, nervous system, lung and skin. Gene therapy is used to treat a variety of conditions including neurodegenerative diseases, lung disorders and gastrointestinal tract disorders. Endogenous EMP-1 expression may be decreased by using anti-sense nucleic acids to a portion of the EMP-1 coding region or to a control region operably linked thereto. The anti-sense nucleic acids may be the full-length EMP-1 gene or to a fragment thereof which hybridizes to the endogenous

EMP-1 gene or a control region regulating expression of same. Anti-sense therapy is used to treat conditions resulting from overexpression of EMP-1 and include neurodegenerative diseases, cancer, lung disorders and gastrointestinal tract disorders.

Nucleic acid sequences of the invention are also used to produce recombinant EMP-1 as described below.

Recombinant production of EMP-1

The invention provides for the recombinant production of EMP-1 in modified host cells. The invention provides materials for carrying out the invention, namely nucleic acid sequences encoding EMP-1, expression vectors containing EMP-1 sequences for producing protein, and modified host cells harboring EMP-1 expression vectors.

The nucleic acid molecules encoding EMP-1 are inserted into expression vectors and introduced into host cells using standard techniques. A suitable expression vector is any one which is capable of expressing EMP-1 in a host cell. It is preferred that an expression vector produces EMP-1 in a mammalian host cell. The modified host cells (i.e, those cells that contain EMP-1 nucleic acid sequences in an appropriate expression vector) are cultured under conditions which favor expression of EMP-1. The host cells of the invention may be any cells which support EMP-1 expression. In a preferred embodiment, the host cells will allow insertion of recombinant EMP-1 into the membrane in a functional configuration. A functional configuration of EMP-1 is characterized by proper insertion into the host cell membrane such that activation of EMP-1 occurs upon contacting a molecule

capable of activation. An example of one such configuration is shown in Figure 4B. It is anticipated that the host cells will typically be mammalian cells. Preferably, host cells will be COS7, CHOd$^-$, NIH 3T3, 293 or 32D cells.

Recombinant production of EMP-1 and display of the recombinant product on host cells are useful for evaluating candidate substances for their ability to bind to EMP-1 and effect a biological response upon formation of a complex with EMP-1. The host cells are used to screen for ligands of EMP-1. The host cells may also be used in screening procedures to identify peptide and small molecule effectors of EMP-1 and proteins which interact with EMP-1.

Screening for EMP-1 agonists and antagonists

An EMP-1 agonist is defined as a substance or compound which stimulates the biological activity of EMP-1. An EMP-1 antagonist is defined as a substance or compound which decreases or inhibits the biological activity of EMP-1 in the presence of a stimulating compound. In general, screening procedures for EMP-1 agonists and antagonists involve contacting candidate substances with EMP-1 bearing host cells under conditions favorable for binding and measuring the extent of receptor activation (in the case of an agonist) or decrease in receptor activation (in the case of an antagonist).

EMP-1 activation may be measured in several ways. Typically, EMP-1 activation is apparent by a change in cell physiology such as an increase or decrease in growth rate, or by a change in differentiation state, or by a change in cell metabolism which can be detected in a microphysiometer.

Antibodies

As described in Example 4, antibodies were generated to peptides from both the mouse and the rat extracellular domains. Antibodies to human EMP-1 may be generated by a similar procedure.

Antibodies specifically recognizing EMP-1 are encompassed by the invention. Antibodies are raised to the extracellular domain of EMP-1 using standard immunological techniques. The antigen may be the intact extracellular domain of membrane-bound EMP-1 or synthetic peptides comprising a portion of the extracellular domain. Antibodies may be polyclonal or monoclonal or may be produced recombinantly, such as for a humanized antibody. An antibody fragment which retains the ability to interact with EMP-1 is also provided. Such a fragment is produced by proteolytic cleavage of a full-length molecule or produced by recombinant DNA procedures. Antibodies of the invention are useful in diagnostic and therapeutic applications. They are used to detect and quantitate EMP-1 in biolgical samples, particuarly tissue samples. They may also be used to modulate the activity of EMP-1 by acting as an agonist or an antagonist.

The rat EMP-1 cDNA sequence as shown in Figure 1 (SEQ ID NO:1) was deposited with the American Type Culture Collection, Rockville, MD on ____________ under accession no. ______________. The mouse EMP-1 cDNA sequence as shown in Figure 2 (SEQ ID NO:3) was deposited with the American Type Culture Collection, Rockville, MD on _______ under accession no. _________. The vector λgt10 containing the human EMP-1 cDNA sequence as shown in Figure 3 (SEQ ID NO:5) was

deposited with the American Type Culture Collection, Rockville, MD on __________ under accession no. _______

The following examples are offered to more fully illustrate the invention, but are not construed as limiting the scope thereof.

EXAMPLE 1

Cloning and Sequencing of EMP-1 cDNAs

During a fetal rat intestine cDNA sequencing project, a 1003 base pair cDNA containing an open reading frame of 480 nucleotides was identified (Fig. 1). The predicted EMP-1 polypeptide of 160 amino acids residues has a calculated molecular weight of approximately 18 kDa. Computer-assisted analysis using the GCG software package reveals that amino acid residues 1-28, 64-89, 95-117 and 134-157 represent four hydrophobic, potentially membrane-spanning domains (Fig. 4C). The amino-terminal 16 amino acids have the characteristics of a signal peptide including a signal peptidase cleavage site after alanine-16 (Fig. 1). Furthermore, a single consensus sequence for N-linked glycosylation is present at asparagine-43 (Fig. 1, Fig. 4B).

EXAMPLE 2

Comparison of EMP-1 and PMP22 DNA Sequences

Comparison of the EMP-1 cDNA sequence to the GenBank database identified PMP22 as the closest known relative with a nucleotide identity of 58% over the open

reading frame. Both the predicted EMP-1 protein and PMP22 are polypeptides of 160 amino acids which show 40% amino acid identity (Fig. 4A). The putative four membrane-spanning regions of EMP-1 and PMP22 are particularly well conserved. The first and second of these hydrophobic domains exhibit the highest degree of amino acid identity at 54% and 67%, respectively, while the third and fourth are only 30% and 37% identical.

Figure 4B depicts a theoretical model of the EMP-1 protein structure based on the hydrophobicity profile and the suggested structure of PMP22. Filled circles represent identical amino acid residues that are shared by rat EMP-1 and rat PMP22 while divergent residues are shown as open circles. The positions of the amino acids in PMP22 known to cause hereditary motor and sensory neuropathies (Suter et al., Hum. Mutation 3, 95-102 (1994) when mutated are highlighted in the EMP-1 sequence as diamonds (Fig. 4B). Interestingly, all of these mutations lie within the putative membrane-spanning domains and five of the six residues are conserved in rat EMP-1. The conservation of these amino acid residues suggests that they may be of functional significance.

Additional database searches with the EMP-1 and PMP22 sequences revealed that both display 30% amino acid identity to the lens fiber cell protein MP20 (Kumar et al. Exp. Eye Res. 56, 35-43 (1993)). MP20 is a 173 amino acid protein with similar structural features to EMP-1 and PMP22 (Fig. 4C). If MP20 is compared to PMP22 and EMP-1 simultaneously, the amino acid identity increases to 36% including strongly conserved motifs in the putative transmembrane domains (Fig. 4C).

EXAMPLE 3

In vitro Transcription and Translation of EMP-1

Both EMP-1 cDNA shown in Figure 1 and mouse PMP22 cDNA (Suter et al. Proc. Natl. Acad. Sci. USA 89, 4382-4386 (1992)) were cloned into the pcDNA-1 expression vector (Invitrogen) downstream of the bacteriophage T7 promoter region and used for *in vitro* transcription/translation assays . mRNA was produced from 0.5 µg of DNA with T7 polymerase and translated *in vitro* in the presence or absence of canine microsomal membranes (CMM) using a reticulocyte lysate system (TNT, Promega). The translation products were labelled metabolically by including [^{35}S]methionine in the reaction. The specificity of the reaction was tested using the vector pcDNA-1 as a control. The efficiency of the CMM was tested with the control reagents α-factor (glycosylation) and prolactin (signal peptide cleavage) according to the manufacturers instructions (TNT, Promega). One tenth of the translation product was denatured in 1% SDS and incubated for 4 hours at 37°C with 1 unit N-Glycosidase F in 50 mM sodium phosphate (pH 7.2), 12.5 mM EDTA, 2.5 mM sodium azide, 25% glycerol and 0.2% SDS. The proteins were separated by reducing 15% SDS PAGE and the gels were subsequently fixed 30 minutes in 50% methanol, 10% acetic acid and treated with enhancer (NEF-981G, Dupont). After drying the gels were exposed to X-ray film (RX, Fuji) overnight at -70°C.

The translation product of EMP-1 cDNA in the absence of canine microsomal membranes (CMM) has an apparent molecular weight of approximately 18 kDa which is in agreement with the calculated molecular weight of

the EMP-1 protein. Translation in the presence of CMM results in a 4-6 kDa increase in molecular weight consistent with the presence of the single putative N-linked glycosylation site in the EMP-1 amino acid sequence (Fig. 5). This migrational shift can be reversed by deglycosylating the translation product with N-Glycosidase F (Fig. 5). The deglycosylated protein migrates identically to the unglycosylated EMP-1 protein suggesting that the putative N-terminal signal peptide is not removed during EMP-1 protein biosynthesis. A similar modification of PMP22 was seen with CMM confirming previous reports of endogenous PMP22 carrying an uncleaved signal peptide (Kitamura et al. in Proceedings of the 6th International Symposium on Glycoconjugates (Yamana, T. et al. eds) pp. 273-274, Jpn. Sci. Soc. Press, Tokyo (1981)).

EXAMPLE 4

Antibodies Reactive with Rat EMP-1

Anti-peptide antibodies were raised to synthetic peptides representing regions of the first and second putative extracellular loops of EMP-1:

Loop1: ^{50}Asp-Gly-Ser-Leu-Ser-Tyr-Gly-Asn-Asp-Asp-Ala-Iso-Lys-Ala^{63}-Cys-COOH

Loop2: ^{116}Tyr-Thr-His-His-Tyr-Ala-His-Ser-Glu-Gly-Asn-Phe-Phe-Pro-Ser-Ser-His-Gln-Gly-Tyr-Cys^{136}-COOH

Amino acids are numbered according to the cDNA predicted polypeptide shown in Figure 1. A C-terminal cysteine residue was added to the loop1 peptide for coupling purposes. The peptides were coupled to keyhole limpet

hemocyanin as described previously (Snipes et al. J. Cell Biol. 117, 225-238 (1992)). The conjugates were used to immunize New Zealand white rabbits with Freund's complete adjuvant and the animals were boosted 4 times with 500 µg peptide and incomplete adjuvant at 2 week intervals. Blood was taken from the animals and serum isolated. The activity of the immune serum was tested on the immunogen by solid-phase ELISA.

EXAMPLE 5

Expression Patterns of EMP-1 mRNA

A. Tissue distribution of EMP-1 and PMP22 mRNA.

To elucidate the distribution of EMP-1 mRNA in the rat, EMP-1 cDNA (Fig. 1) was used to probe Northern blots of total RNA extracted from various tissues.

RNA isolation and Northern blot analysis was carried out as follows. Total RNA was extracted from rat tissues using a modified acid phenol method. Briefly, tissues were homogenised into GT buffer (Chomczynski et al. Anal. Biochem. 162, 156-159 (1987)). The lysate was cleared and extracted twice with phenol/chloroform (1:1). The RNA was precipitated, resuspended in diethyl pyrocarbonate-treated H_2O and quantified by OD_{260}/OD_{280} measurement. Ten µg of total RNA was loaded onto denaturing 1.2% agarose formaldehyde gels. Separated RNA was transferred to nylon membrane (Hybond N, Amersham) by capillarity and cross-linked with 240 mJ of UV irradiation in a Stratalinker (Stratagene). Equal loading and transfer to the membrane was assessed by ethidium bromide staining. Membranes were prehybridized for 6 hours and hybridized 36 hours at 42°C in a solution containing 50% formamide. cDNA fragments of PMP22 and EMP-1 containing the entire open

reading frames were labelled with ^{32}P-dCTP by random hexamer priming (Oligolabelling kit, Pharmacia). Northern blots were washed at high stringency and exposed to X-ray film (RX, Fuji) for 12 to 72 hours.

EMP-1 transcripts can be found in all organs examined with the exception of the liver (Fig. 6a and b). The most prominent EMP-1 mRNA expression is observed in tail-derived skin and in the gastrointestinal tract. To examine a potential specific regional expression pattern, RNA was extracted from different regions of the gastrointestinal tract. Interestingly, we found that EMP-1 mRNA is not uniformly expressed throughout the stomach of the rat. The fundic region exhibits high levels of EMP-1 mRNA while expression in the corpus and pylorus are much lower. In the intestinal tract, the caecum and large intestine (colon and rectum) contain the highest levels of EMP-1 mRNA. EMP-1 transcripts are also detectable throughout the small intestine, but at far lower levels than in the fundus of the stomach, the caecum and large intestine. Considerable amounts of EMP-1 mRNA, similar to the expression in the duodenum, are also found in the brain and lung. Low-level EMP-1 expression is detectable in the heart, kidney, spleen, thymus and skeletal muscle.

All tissues expressing EMP-1 mRNA contain 2.8 kb EMP-1 transcripts. In some regions of the gastrointestinal tract, however, including the fundus, ileum, caecum and colon, additional transcripts of approximately 1.7 kb hybridize with the EMP-1 cDNA (Fig. 6a). Prolonged washing of the blots at high stringency did not result in the preferential loss of one of the signals relative to the other, hence, we favour the interpretation that both the 2.8 kb and 1.7 kb transcripts are derived from the EMP-1 gene. Further

studies of the differently sized transcripts will determine if they result from the use of alternative polyadenylation sites or arise by alternative splicing.

The EMP-1-probed Northern blot was stripped and re-probed with labelled rat PMP22 cDNA (Fig. 6c and d). The results show that the tissue distribution of PMP22 mRNA and EMP-1 mRNA is similar, but that there are subtle differences in their relative expression levels. PMP22 and EMP-1 transcripts are co-expressed to high levels in the skin, fundus of the stomach, caecum, colon, rectum and duodenum. However, while the EMP-1 mRNA level is relatively high in colon compared to the rectum, PMP22 mRNA is low. Furthermore, PMP22 mRNA is more prominently expressed in the lung than EMP-1 mRNA but is relatively underrepresented in the brain (Fig. 6). Neither EMP-1 or PMP22 transcripts could be detected by Northern analysis of liver RNA.

B. <u>Regulation of EMP-1 and PMP22 expression after sciatic nerve injury</u>. The highest levels of PMP22 are found in myelinating Schwann cells of the PNS and expression is down regulated in the distal portion of the rat sciatic nerve following crush or cut injury Welcher et al. Proc. Natl. Acad. Sci. USA. <u>88</u>, 7195-7199 (1991); Spreyer et al. EMBO J. <u>10</u>, 3661-3668 (1991); Snipes et al. J. Cell Biol. <u>117</u>, 225-238 (1992); De Leon et al. J. Neurosci. Res. <u>29</u>, 437-448 (1991). EMP-1 mRNA levels in the sciatic nerve were examined under the same conditions. The sciatic nerves of male SIV rats (8-wk old; University of Zurich, Switzerland) were bilaterally exposed and cut unilaterally. Four days after injury, the degenerating distal portion of the traumatized nerve and, as a control, the undamaged contralateral nerve were removed and total RNA was isolated.

Northern blot analysis reveals that EMP-1 transcripts are present at considerably lower levels in the adult sciatic nerve than PMP22 transcripts; Figure 7A represents exposures of 1 hour for PMP22 and approximately 36 hours for EMP-1. Furthermore, EMP-1 expression increases in the distal nerve following injury, in sharp contrast to PMP22 (Fig. 7A). These findings demonstrate that, although EMP-1 and PMP22 are coexpressed in PNS nerves, they appear to be differently regulated.

C. Regulation of EMP-1 and PMP22 expression in Schwann Cells in vitro. EMP-1 and PMP22 mRNA levels were compared in mitogen-expanded primary rat Schwann cells (pSC) and D6P2T Schwann cells grown in the presence or absence of forskolin. Forskolin has been shown to induce the expression of PMP22 and other myelin proteins in cultured Schwann cells via a mechanism proposed to partially mimic axon-Schwann cell interactions that occur during myelination Spreyer et al., supra; Pareek et al. J. Biol. Chem. 268, 10372-10379 (1993); Lemke et al. Development. 102, 499-504 (1988).

Rat Schwann cells were isolated from the sciatic nerve of neonatal rats using the method of (Brockes et al. Brain Res. 165, 105-118 (1979)) with modifications as described previously (Pareek et al., supra). The mitogen-expanded primary rat Schwann cells were cultured on poly-L-lysine coated culture plates in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal calf serum (FCS), 20 µg/ml crude glial growth factor, 5 µg/ml forskolin and 50 µg/ml gentamicin (Pareek et al., supra). The Schwann cell derived cell line, D6P2T (Bansal et al. J. Neurochem. 49, 1902-1911

(1987)) was grown in plastic culture dishes in DMEM containing 5% FCS and 50 µg/ml gentamicin. The cells were split into two groups and cultured in the same medium without forskolin. After 3 days, the cells had reached 70% confluency, one group of cells were treated with 20 µg/ml forskolin for 36 hours, while the second was maintained in the absence of forskolin. Subsequently, the cells were harvested in 5 M GT buffer and total RNA was extracted. PMP22 mRNA is upregulated by forskolin in pSC and D6P2T cells (Fig. 7B). In contrast, EMP-1 mRNA levels are decreased under the same conditions. This regulation is particularly prominent in the D6P2T cell line where EMP-1 mRNA is reduced in the presence of forskolin to barely detectable levels.

D. <u>Regulation of EMP-1 and PMP22 after growth arrest in NIH3T3 Cells</u>. NIH 3T3 fibroblasts were cultured and growth arrested by serum deprivation as described previously (Suter et al. J. Biol. Chem. <u>269</u>, 1-14 (1994)). Exponentially growing and growth arrested cells were harvested into 5 M GT buffer. Under these conditions, NIH3T3 fibroblasts exhibited increased PMP22 mRNA expression (Fig. 7C). Manfioletti et al. Mol. Cell. Biol. <u>10</u>, 2924-2930 (1990). In contrast to the regulation of PMP22, EMP-1 mRNA levels are strongly decreased under identical experimental conditions (Fig. 7C).

E. <u>Expression of EMP-1 protein in the rat intestine</u>. Rat tissues were homogenised into 8 M urea and cleared at 10,000 g for 10 min at 4°C. The protein concentration of the supernatant was assessed by Bradford assay and 50 µg of protein were denatured by heating to 95°C for 3 minutes in sample buffer

containing 2% β-mercaptoethanol and loaded on a 12% SDS-PAGE. The proteins were electrotransferred to nitrocellulose membrane (Schleicher & Schuell) using a semi-dry blotter (Bio-Rad). Membranes were stained with Ponceau S to test transfer efficiency. Blots were blocked with 0.15% casein in phosphate buffered saline containing 0.2% Tween20 (Sigma), incubated with the polyclonal rabbit sera at a dilution of 1:500 in blocking buffer followed by a 1 hour incubation with Horse Radish Peroxidase-labelled goat anti-rabbit immunoglobulin (1:5000, Sigma). Detection was by chemiluminescence (ECL, Amersham) and exposure of X-ray film (RX, Fuji).

Both anti-loop 1 and anti-loop 2 antibodies recognize a protein of approximately 25 kDa by Western blotting of various gastrointestinal tract tissue lysates (Fig. 8) but the anti-loop1 antibodies are considerably more efficient. The labelling of immunoreactive proteins on Western blots can be blocked by preincubation of the antiserum in the presence of 250 µg/ml of immunogen peptide confirming specificity of the signal (Fig. 8A). Blocking is specific for the immunogen and is not effected by preincubation with the same concentration of a different peptide (Fig. 8A). The calculated molecular weight of the core EMP-1 protein is approximately 18 kDa which can be confirmed by *in vitro* transcription and translation of the cDNA (Fig. 5). The presence of a putative N-linked carbohydrate chain conceivably results in a protein with an apparent molecular weight of 25 kDa on reducing SDS-PAGE. However, additional post-translational modifications of the EMP-1 protein cannot be excluded.

The most prominent expression of EMP-1 protein is seen in the stomach with lower levels being

detectable in the caecum and large intestine. Expression in the duodenum and jejunum of the small intestine is considerably lower than in the other regions of the intestinal tract in accordance with the reduced mRNA levels found in these tissues (Fig. 6, 8A). Very low levels of the 25 kDa EMP-1 protein can also be detected in the lung (Fig. 8A), spleen and thymus.

In addition to the 25 kDa protein, both EMP-1 antisera detect a similar array of larger proteins in the intestine (Fig. 8B). The presence of these additional immunoreactive species varies from experiment to experiment and between tissues. In general, the additional bands are most prominent in lysates containing higher amounts of EMP-1 protein. Since the two antisera are directed against independent regions of EMP-1 protein, these larger immunoreactive species are likely to represent aggregated molecules, a phenomenon frequently seen with highly hydrophobic proteins.

Although the level of EMP-1 protein observed in some tissues does not strictly correlate with EMP-1 mRNA expression, EMP-1 protein can only be found in tissues where EMP-1 mRNA expression is seen. No immunoreactive proteins are detected by either antiserum in lysates of the EMP-1 mRNA-negative liver (Fig. 8A).

EXAMPLE 6

Expression of Recombinant EMP-1

A. Transient Expression of Recombinant Rat EMP-1.

The EMP-1 cDNA shown in Figure 1 was subcloned into the EcoRV site of the pcDNA1 (InVitrogen) expression vector, down stream of the cytomegalovirus (CMV) promoter. The parent vector without cDNA insert was used in the negative control transfections.

Recombinant DNA was purified by QIAGEN column isolation and quantitated by OD_{260}. COS cells exponentially growing in DMEM containing 10% FCS were trypsinised, washed in phosphate buffered saline and pelleted at 800 g. $1.5x10^6$ cells were resuspended in 200 µl phosphate buffered saline containing 5 µg of vector DNA. The cells were chilled on ice for 5 minutes, transferred to a 4 mm gap electroporation cuvette (Bio-Rad) and electroporated with 300 Volts and 125 µFarads. The transfected cells were chilled for 5 minutes on ice and split into seven 35 mm culture dishes containing 2 ml of DMEM and 10% FCS and cultured for 48 hours. The cells were then washed with Tris-buffered saline, fixed in DMEM containing 2% paraformaldehyde for 30 minutes, washed with Tris-buffered saline and permeabilized for 30 minutes in Tris-buffered saline containing 0.1% saponin. Unspecific binding sites were blocked for 30 minutes at room temperature in Tris-buffered saline, 2% bovine serum albumin, 0.1% porcine skin gelatin (type A Sigma), 2% goat serum and 0.1% saponin. The cells were incubated with the anti-EMP-1 antibodies diluted 1:500 in blocking buffer containing 0.02% saponin overnight at 4°C followed by FITC-labelled goat anti-rabbit immunoglobulin (Cappel) at 1:200 in blocking buffer with 0.02% saponin. After washing with Tris-buffered saline, coverslips were mounted using AF1 (Citifluor) and immunoreactivity visualised by confocal microscopy with a Bio-Rad, MRC-600 scanner in conjunction with a Zeiss Axiophot fluorescence microscope using Imaris image processing software (Bitplane AG, Technopark Zürich, Switzerland). Preimmune serum (1:500) as primary antiserum was used as a negative control.

After COS cell transfection, both anti-loop 1 and anti-loop 2 antisera identified approximately 25% of

the cells with strong immunoreactivity (Fig. 9). Neither antiserum nor preimmune sera recognised control COS cells transfected with the parental expression vector without EMP-1 insert. Since detection with anti-loop2 antiserum was more efficient than with the anti-loop1 antiserum, the former antibody was predominantly used in subsequent studies.

B. Expression of recombinant human EMP-1.

The EMP-1 cDNA sequence as shown in Figure 3 was cloned into pCDNA-1 and transfected into COS cells. COS cells were cultured as in Example 6A and cell lysates were prepared and analyzed for EMP-1 protein expression by Western analysis. The protein is detected by anti-sera to human EMP-1.

EXAMPLE 7

Localization of EMP-1 Protein Expression in Rat Intestine

Figure 10a shows a schematic representation of the topology of the rat gastric mucosa. The epithelial cells of the gastric pit are produced from stem cells in the isthmus/neck region of the gastric gland. These cells differentiate during their migration towards the gastric pit from where they are extruded (exfoliated) from the tip of the vilus (reviewed by Gordon and Hermiston Curr. Opin. Cell. Biol. 6, 795-803 (1994)). Transverse sections across the gastric pit show epithelial cells that are organized in circles around the intestinal lumen (Fig. 10a).

Tissue preparation and immunofluorescence were performed as follows. The stomach was removed from adult SIV rats, cut into blocks 10 mm by 5 mm, and snap

frozen into OCT mounting medium (Tissue Tech) in isopentane on liquid nitrogen. Ten µm frozen sections were cut and thaw mounted onto slides subbed with 0.5% gelatin and 0.05% potassium chromate. The sections were fixed in 1% paraformaldehyde for 5 minutes and washed in phosphate buffered saline. Unspecific binding sites were blocked for 1 hour with goat serum diluted 1:50 in phosphate buffered saline. Sections were incubated overnight at 4°C or 4 hours at room temperature with 1:500 dilution of primary antiserum in phosphate buffered saline containing 0.2% Tween20 followed by 2 hours with 1:200 dilution of Texas Red-labelled donkey anti-rabbit immunoglobulin (Jackson Immunoresearch Laboratories). After extensive washing in phosphate buffered saline containing 0.2% Tween20, sections were coated with AF1 (Citifluor) and cover-slips mounted. Immune reactivity was visualised by high resolution confocal microscopy. Preimmune serum (1:500) as primary antiserum was used as a negative control.

Ten µm frozen sections of corpus gastricum were stained with anti-loop2 antiserum. Strong immunoreactivity was detected in the outer epithelial cells of the gastric mucosa from the tip of the vilus down towards the isthmus and neck of the gastric gland (Fig. 10b, c). In transverse section, the EMP-1 immunoreactivity appears to be associated with the plasmamembrane of epithelial cells in the gastric pits (Fig. 10e). The epithelial cells deeper in the gastric gland show little or no immunoreactivity and specific labelling was not detectable in the base of the gastric gland or in the sub-mucosal muscle layer (Fig. 10b).

* * *

While the invention has been described in what is considered to be its preferred embodiments, it is not to be limited to the disclosed embodiments, but on the contrary, is intended to cover various modifications and equivalents included within the spirit and scope of the appended claims, which scope is to be accorded the broadest interpretation so as to encompass all such modifications and equivalents.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Amgen Inc.

(ii) TITLE OF INVENTION: EPITHELIAL MEMBRANE PROTEIN-1

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Amgen Inc.
(B) STREET: 1840 Dehavilland Drive
(C) CITY: Thousand Oaks
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 91320-1789

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/563,839
(B) FILING DATE: 23-NOV-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Winter, Robert B.
(C) REFERENCE/DOCKET NUMBER: A-366

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1003 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 132..611

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Lys Ala Val Gln Ala Phe Met Ile Leu Ser Ile Ile Phe Ser Ile Ile
             65                  70                  75

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         80                  85                  90

CGG TTC TTC CTC TCG GGA TCC ACC ATG CTG GTG TGC TGG CTG TGC ATT      458
Arg Phe Phe Leu Ser Gly Ser Thr Met Leu Val Cys Trp Leu Cys Ile
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CTG ATT GGA GTG TCT ATC TAC ACT CAC CAC TAC GCC CAC AGC GAA GGG      506
Leu Ile Gly Val Ser Ile Tyr Thr His His Tyr Ala His Ser Glu Gly
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 160 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Cys Asp Gly Ser Leu Ser Tyr Gly Asn Asp Asp Ala Ile Lys Ala Val
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Gln Ala Phe Met Ile Leu Ser Ile Ile Phe Ser Ile Ile Ser Leu Val
 65                  70                  75                  80

Val Phe Val Phe Gln Leu Phe Thr Met Glu Lys Gly Asn Arg Phe Phe
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Val Ser Ile Tyr Thr His His Tyr Ala His Ser Glu Gly Asn Phe Phe
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Pro Ser Ser His Gln Gly Tyr Cys Phe Ile Leu Thr Trp Ile Cys Phe
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2323 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 183..662

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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                 20                  25                  30

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         50                  55                  60

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     65                  70                  75

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 80                  85                  90                  95

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Phe Leu Ser Gly Ser Thr Met Leu Val Cys Trp Leu Cys Ile Leu Val
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TTC TGT TTC AGC TTC ATC ATC GGC ATA CTC TAT ATG GTC CTG AGG AAG       659
Phe Cys Phe Ser Phe Ile Ile Gly Ile Leu Tyr Met Val Leu Arg Lys
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Lys
160

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GGTGGGATTC TCTACTGCCA AGCACCCATC CTTGGAAGAA AGTTGTTGGC TGATATGCTG     892
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ATGCTTCCTT GACGTCACCA GAGAGTCCTC CTCTAGCCAC CAAATATGGC CCCGTCCATC 952

CTCAATTACA TACACTCGGG GCCTCCCCAG CTGCCATACC ACTGGCGCCA CTCTTGAGGG 1012

TGGCTGCTGG GTCACACACT GAGGTCTTCC ACATCCCATA TCATCAAGTT CTGATGGTGG 1072

TTCAGGTCTT AGCAAGAGCA GATATTGCTC GATGCTGAGG CTAAGTCTGG AAGCCACTTT 1132

GTCCTTGTGA CCTAAAACCA AACATCAAAT CCAGATCCCA TGTGCCTGTA GTGGGAGCTT 1192

TGGCCAGGAA GCCAATGTGC ATATTTGGTG GCCTTTCTAA CAAAAGTATA GGATGATGAG 1252

AGATGGTTTG TAAGTTCAAA GCTGATGGAA TTGGTTTAGC CAAGAAATGG AAGTTTCTAC 1312

CCCAGAGGAT CCTTGGAGAC AGGTGGGGAC AGGCAGTGCT CCTCAGTCAC GTGTCACCGA 1372

GCTGTCCCTC ATGGAGGCCT CCTGTTGTGA ACTCTGCTAG ACTCTCACTT ACAGCCAAGG 1432

CAGCTTTTCT GGAGTTTTTC TTAGATTCTC TAGAGCCAAA GATGATAATG CCTCACAAAA 1492

CATAGGGTCA AAGCATATGC CCACCGCAGT GCTATAGTAA GTTTGTGGGT TTTTAGGATT 1552

CCCCCAAAGC ACTCAATGTA TCTTGTATAT GTAACAGGGG AGAAATGCAT GTGTTCCTTT 1612

GACATACAAT TCTGAACTAG GAATATTTGA GGAAGTCCAA TGATGACCAA CAACACTGGG 1672

GACCAGAATA TAACATCTAA ATGCAGTAGT CACTGTTGCT TTGACCTGGG CTGGAGTGGT 1732

CTCCTCTCAA CAGCTTTCAT CACACTATTT TCCAGCTAAA GATGGCAAAG CTGTAAGCCA 1792

ATTAACATAT ACACCAACCT AAACTAAAGA ACCAGTCCTG AGGGTGTGAG AAAGGTGCTA 1852

TCTGGTTATG GATTATTAAG CAAACCATAT TTCATTTATG TTGAGAAGAG AATGCCTGCC 1912

CTCAGGGAAA AAAAAATGTA ATTGTGTGAG ATGAATAAAG TCCTGGTGAT AGGCAGACAG 1972

TTTCTTTTTT AAAACAGGAG AAACTCTTAG GGCATCCAGA CAGATGGTAG CTAAATTGTT 2032

GGGGCTGCAG GGGTATTCCT GTATAAGACT TAGAGGTAGT ATGATATCTC AGATTTCTGC 2092

CTTAAAGGGC TTTCTTTTTA GAAATAGTTT CTTTTATTGC CCTTAGAAGA TCACCCCCAG 2152

GAAGAGTATG AGCTATCTTT TCTACATTTC TTTTCCTAGG AATATTCTTA TCCATTTCTT 2212

ATATACATTT CTTTTGGGAG GGAGTTTTTA TGCTATAGTT GCTGGTATTT ATGTAAAGGG 2272

ACCATTACTA AGTGTATTTC TCTAGCATAT TATGTTTAAG GGACGTGTGT A 2323

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 160 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Leu Val Leu Leu Ala Gly Leu Phe Val Val His Ile Ala Thr Ala
 1               5                  10                  15

Ile Met Leu Phe Val Ser Thr Ile Ala Asn Val Trp Met Val Ala Asp
            20                  25                  30

Tyr Ala Asn Ala Ser Val Gly Leu Trp Lys Asn Cys Thr Gly Gly Asn
        35                  40                  45

Cys Asp Gly Ser Leu Ser Tyr Gly Asn Glu Asp Ala Ile Lys Ala Val
    50                  55                  60

Gln Ala Phe Met Ile Leu Ser Ile Ile Phe Ser Ile Ile Ser Leu Val
 65                  70                  75                  80

Val Phe Val Phe Gln Leu Phe Thr Met Glu Lys Gly Asn Arg Phe Phe
                 85                  90                  95

Leu Ser Gly Ser Thr Met Leu Val Cys Trp Leu Cys Ile Leu Val Gly
            100                 105                 110

Val Ser Ile Tyr Thr His His Tyr Ala His Ser Glu Gly Asn Phe Asn
        115                 120                 125

Ser Ser Ser His Gln Gly Tyr Cys Phe Ile Leu Thr Trp Ile Cys Phe
    130                 135                 140

Cys Phe Ser Phe Ile Ile Gly Ile Leu Tyr Met Val Leu Arg Lys Lys
145                 150                 155                 160
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1725 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 8..478

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
AGCCAAC ATG TTG GTA TTG CTG GCT GGT ATC TTT GTG GTC CAC ATC GCT           49
        Met Leu Val Leu Leu Ala Gly Ile Phe Val Val His Ile Ala
         1               5                  10

ACT GTT ATT ATG CTA TTT GTT AGC ACC ATT GCC AAT GTC TGG TTG GTT           97
Thr Val Ile Met Leu Phe Val Ser Thr Ile Ala Asn Val Trp Leu Val
 15                  20                  25                  30
```

TCC AAT ACG GTA GAT GCA TCA GTA GGT CTT TGG AAA AAC TGT ACC AAC 145
Ser Asn Thr Val Asp Ala Ser Val Gly Leu Trp Lys Asn Cys Thr Asn
35 40 45

ATT AGC TGC AGT GAC AGC CTG TCA TAT GCC AGT GAA GAT GCC CTC AAG 193
Ile Ser Cys Ser Asp Ser Leu Ser Tyr Ala Ser Glu Asp Ala Leu Lys
50 55 60

ACA GTG CAG GCC TTC ATG ATT CTC TCT ATC ATC TTC TGT GTC ATT GCC 241
Thr Val Gln Ala Phe Met Ile Leu Ser Ile Ile Phe Cys Val Ile Ala
65 70 75

CTC CTG GTC TTC GTG TTC CAG CTC TTC ACC ATG GAG AAG GGA AAC CGG 289
Leu Leu Val Phe Val Phe Gln Leu Phe Thr Met Glu Lys Gly Asn Arg
80 85 90

TTC TTC CTC TCA GGG GCC ACC ACA CTG GTG TGC TGG CTG TGC ATT CTT 337
Phe Phe Leu Ser Gly Ala Thr Thr Leu Val Cys Trp Leu Cys Ile Leu
95 100 105 110

GTG GGG GTG TCC ATC TAC ACT AGT CAT TAT GCG AAT CGT GAT GGA ACG 385
Val Gly Val Ser Ile Tyr Thr Ser His Tyr Ala Asn Arg Asp Gly Thr
115 120 125

CAG TAT CAC CAC GGC TAT TCC TAC ATC CTG GGC TGG ATC TGC TTC TGC 433
Gln Tyr His His Gly Tyr Ser Tyr Ile Leu Gly Trp Ile Cys Phe Cys
130 135 140

TTC AGC TTC ATC ATC GGC GTT CTC TAT CTG GTC CTG AGA AAG AAA 478
Phe Ser Phe Ile Ile Gly Val Leu Tyr Leu Val Leu Arg Lys Lys
145 150 155

TAAGGCCGGA CGAGTTCATG GGGATCTGGG GGGTGGGGAG GAGGAAGCCG TTGAATCTGG 538

GAGGGAAGTG GAGGTTGCTG TACAGGAAAA ACCGAGATAG GGGAGGGGGG AGGGGGAAGC 598

AAAGGGGGGA GGTCAAATCC CAAACCATTA CTGAGGGGAT TCTCTACTGC CAAGCCCCTG 658

CCCTGGGGAG AAAGTAGTTG GCTAGTACTT TGATGCTCCC TTGATGGGGT CCAGAGAGCC 718

TCCCTGCAGC CACCAGACTT GGCCTCCAGC TGTTCTTAGT GACACACACT GTCTGGGGCC 778

CCATCAGCTG CCACAACACC AGCCCCACTT CTGGGTCATG CACTGAGGTC CACAGACCTA 838

CTGCACTGAG TTAAAATAGC GGTACAAGTT CTGGCAAGAG CAGATACTGT CTTTGTGCTG 898

AATACGCTAA GCCTGGAAGC CATCCTGCCC TTCTGACCCA AAGCAAAACA TCACATTCCA 958

GTCTGAAGTG CCTACTGGGG GGCTTTGGCC TGTGAGCCAT TGTCCCTCTT TGGAACAGAT 1018

ATTTAGCTCT GTGGAATTCA GTGACAAAAT GGGAGGAGGA AAGAGAGTTT GTAAGGTCAT 1078

GCTGGTGGGT TAGCTAAACC AAGAAGGAGA CCTTTTCACA ATGGAAAACC TGGGGGATGG 1138

TCAGAGCCCA GTCGAGACCT CACACACGGC TGTCCCTCAT GGAGACCTCA TGCCATGGTC 1198

TTTGCTAGGC CTCTTGCTGA AAGCCAAGGC AGCTCTTCTG GAGTTTCTCT AAAGTCACTA 1258

GTGAACAATT CGGTGGTAAA AGTACCACAC AAACTATGGG ATCCAAGGGG CAGTCTTGCA 1318

ACAGTGCCAT GTTAGGGTTA TGTTTTTAGG ATTCCCCTCA ATGCAGTCAG TGTTTCTTTT 1378

AAGTATACAA CAGGAGAGAG ATGGACATGG CTCATTGTAG CACAATCCTA TTACTCTTCC 1438

TCTAACATTT TTGAGGAAGT TTTGTCTAAT TATCAATATT GAGGATCAGG GCTCCTAGGC 1498

TCAGTGGTAG CTCTGGCTTA GACACCACCT GGAGTGATCA CCTCTTGGGG ACCCTGCCTA 1558

TCCCACTTCA CAGGTGAGGC ACCGGAATTC TGGAAGCTGA TTAAAACACA CATAAACCAA 1618

AACCAAACAA CAGGCCCTTG GGTGAAAGGT GCTATATAAT TGTGAAGTAT TAAGCCTACC 1678

GTATTTCAGC CATGATAAGA ACAGAGTGCC TGCATTCCCA GGAAAAT 1725

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 157 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Leu Val Leu Leu Ala Gly Ile Phe Val Val His Ile Ala Thr Val
1 5 10 15

Ile Met Leu Phe Val Ser Thr Ile Ala Asn Val Trp Leu Val Ser Asn
20 25 30

Thr Val Asp Ala Ser Val Gly Leu Trp Lys Asn Cys Thr Asn Ile Ser
35 40 45

Cys Ser Asp Ser Leu Ser Tyr Ala Ser Glu Asp Ala Leu Lys Thr Val
50 55 60

Gln Ala Phe Met Ile Leu Ser Ile Ile Phe Cys Val Ile Ala Leu Leu
65 70 75 80

Val Phe Val Phe Gln Leu Phe Thr Met Glu Lys Gly Asn Arg Phe Phe
85 90 95

Leu Ser Gly Ala Thr Thr Leu Val Cys Trp Leu Cys Ile Leu Val Gly
100 105 110

Val Ser Ile Tyr Thr Ser His Tyr Ala Asn Arg Asp Gly Thr Gln Tyr
115 120 125

His His Gly Tyr Ser Tyr Ile Leu Gly Trp Ile Cys Phe Cys Phe Ser
130 135 140

Phe Ile Ile Gly Val Leu Tyr Leu Val Leu Arg Lys Lys
145 150 155

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 173 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Tyr Ser Phe Met Gly Gly Gly Leu Phe Cys Ala Trp Val Gly Thr
1               5                   10                  15

Ile Leu Leu Val Val Ala Thr Ala Thr Asp His Trp Met Gln Tyr Arg
            20                  25                  30

Leu Ser Gly Ser Phe Ala His Gln Gly Leu Trp Arg Tyr Cys Leu Gly
        35                  40                  45

Asn Lys Cys Phe Leu Gln Thr Glu Ser Ile Ala Tyr Trp Asn Ala Thr
    50                  55                  60

Arg Ala Phe Met Ile Leu Ser Ala Leu Cys Ala Thr Ser Gly Ile Ile
65                  70                  75                  80

Met Gly Val Leu Ala Phe Ala Gln Gln Ser Thr Phe Thr Arg Leu Ser
                85                  90                  95

Arg Pro Phe Ser Ala Gly Ile Met Phe Phe Ala Ser Thr Leu Phe Val
            100                 105                 110

Leu Leu Ala Leu Ala Ile Tyr Thr Gly Val Thr Val Ser Phe Leu Gly
        115                 120                 125

Arg Arg Phe Gly Asp Trp Arg Phe Ser Trp Ser Tyr Ile Leu Gly Trp
    130                 135                 140

Val Ala Leu Leu Met Thr Phe Phe Ala Gly Ile Phe Tyr Met Cys Ala
145                 150                 155                 160

Tyr Arg Met His Glu Cys Arg Arg Leu Ser Thr Pro Arg

                165                 170
```

(Rev. ... 12/93 Pub N151) FORM 13-27 13-205

Applicant's or agent's file reference number A-366	International application No. Not Yet Assigned

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis)*

A. The indications made below relate to the microorganism referred to in the description on page 18, line 10, 15, 20

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
American Type Culture Collection

Address of depositary institution *(including postal code and country)*
12301 Parlawn Drive
Rockville, MD 20852 USA

Date of deposit	Accession Number
November 28, 1995	97345, 97346 and 97347

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications, e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer Sigfried Hoctad International Division RO/US (703) 305-3080	Authorized officer

Form PCT/RO/134 (July 1992)

(Indications Relating to a Deposited Mocroorganism (Form PCT/RO/134) (13-27)—page 1 of 1)

CLAIMS:

1. An isolated nucleic acid molecule encoding EMP-1.

2. The molecule of Claim 1 which encodes mammalian EMP-1.

3. The molecule of Claim 1 which encodes rat EMP-1 having the sequence shown in Figure 1.

4. The molecule of Claim 1 which encodes mouse EMP-1 having the sequence shown in Figure 2.

5. The molecule of Claim 1 which encodes human EMP-1 having the sequence shown in Figure 3.

6. A DNA molecule comprising an expression system which, when transfected into a host cell, is capable of expressing EMP-1.

7. The DNA molecule of Claim 6 wherein the expression system comprises a nucleotide sequence encoding EMP-1.

8. A host cell modified with the DNA molecule of Claim 6.

9. The host cell of Claim 8 which is a mammalian cell.

10. The host cell of Claim 8 wherein EMP-1 is displayed at the cell surface.

11. An antibody or fragment thereof which specifically binds to EMP-1.

12. The antibody of Claim 11 which is an agonist of EMP-1 activity.

13. The antibody of Claim 11 which is an antagonist of EMP-1 activity.

14. A method of expressing EMP-1 in a host cell comprising:

modifiying the cells with the DNA molecule of Claim 6; and

culturing the cells under conditions which allow the expression of EMP-1.

15. The method of Claim 14 wherein EMP-1 is displayed at the surface of the host cell.

16. A method of altering the expression of an endogenous EMP-1 gene in a selected tissue comprising modifying the tissue with an exogenous EMP-1 nucleic acid sequence wherein the exogenous sequence increases or decreases the tissue level of EMP-1.

17. The method of Claim 16 wherein the exogenous EMP-1 nucleic acid sequence contains the EMP-1 coding region operably linked to a tissue-specific expression system.

18. The method of Claim 16 wherein the exogenous EMP-1 nucleic acid sequence is complementary to a portion of the endogenous EMP-1 gene.

19. A method to assess the ability of a candidate substance to behave as an EMP-1 agonist, which method comprises:

incubating the cells of Claim 10 with the candidate substance under conditions which allow activation of EMP-1; and

measuring the activation of EMP-1 resulting therefrom.

20. A method to assess the ability of a candidate substance to behave as an EMP-1 antagonist, which method comprises:

incubating the cells of Claim 10 with the candidate substance wherein EMP-1 has been activated; and

measuring a subsequent decrease in activation of EMP-1.

21. A method for detecting the presence of EMP-1 in a biological sample comprising:

incubating the sample with the antibody of Claim 11 under conditions allowing binding of the antibody to EMP-1; and

detecting the bound antibody.

22. A method for identifying in a biological sample mutations or alternations in an EMP-1 nucleic acid sequence comprising:

incubating the sample with the nucleic acid of Claim 1, or a portion thereof, under donditions permitting hybridization;

isolating the EMP-1 nucleic acid in the sample; and

identifying the mutation or alteration in the EMP-1 nucleic acid.

FIG.1A

```
GTCGACCCAC GCGTCCGAAA CCTCCTGAAG AGAGGACCAG ACCAGCAGCC AGCGCCACCA    60

CTCAGGGCAT CTGCCTCTGT CACTGGATAC TCCAGAATTC TCTACTCAGG AGTTACAAAA   120

AAGAAGCCAA G ATG TTG GTG CTA CTG GCC GGT CTC TTC GTG GTC CAC ATC    170
             Met Leu Val Leu Leu Ala Gly Leu Phe Val Val His Ile
               1               5                  10

GCC ACT GCC ATT ATG CTG TTT GTC TCC ACC ATT GCC AAC GTC TGG ATG     218
Ala Thr Ala Ile Met Leu Phe Val Ser Thr Ile Ala Asn Val Trp Met
     15                  20                  25

GTG GCG GAC GGT ATA GAC TCG TCC ATA GGG CTT TGG AAG AAC TGC ACC     266
Val Ala Asp Gly Ile Asp Ser Ser Ile Gly Leu Trp Lys Asn Cys Thr
 30                  35                  40                  45

AGT GGC AGC TGT GAC GGC TCT CTG AGC TAC GGC AAT GAT GAT GCT ATC     314
Ser Gly Ser Cys Asp Gly Ser Leu Ser Tyr Gly Asn Asp Asp Ala Ile
                 50                  55                  60

AAG GCA GTG CAA GCT TTC ATG ATC CTC TCC ATC ATC TTC TCT ATA ATC     362
Lys Ala Val Gln Ala Phe Met Ile Leu Ser Ile Ile Phe Ser Ile Ile
             65                  70                  75

TCC CTC GTG GTC TTC GTG TTC CAG CTC TTC ACC ATG GAG AAG GGA AAC     410
Ser Leu Val Val Phe Val Phe Gln Leu Phe Thr Met Glu Lys Gly Asn
         80                  85                  90

CGG TTC TTC CTC TCG GGA TCC ACC ATG CTG GTG TGC TGG CTG TGC ATT     458
Arg Phe Phe Leu Ser Gly Ser Thr Met Leu Val Cys Trp Leu Cys Ile
     95                 100                 105
```

FIG.1B

```
CTG ATT GGA GTG TCT ATC TAC ACT CAC CAC TAC GCC CAC AGC GAA GGG      506
Leu Ile Gly Val Ser Ile Tyr Thr His His Tyr Ala His Ser Glu Gly
110                 115                 120                 125

AAC TTT TTC CCC AGC AGC CAT CAA GGC TAC TGT TTC ATC CTG ACC TGG      554
Asn Phe Phe Pro Ser Ser His Gln Gly Tyr Cys Phe Ile Leu Thr Trp
                130                 135                 140

ATT TGC TTC TGC TTC AGC TTC ATC ATC GGC ATA CTC TAT ATG GTC CTG      602
Ile Cys Phe Cys Phe Ser Phe Ile Ile Gly Ile Leu Tyr Met Val Leu
            145                 150                 155

AGG AAG AAA TAAGCTCGTG GGCATCTGGG GTGGGGGTGG GGAAGTAGGG              651
Arg Lys Lys
        160

AGGAGGAAGC AACTGAATCC GGGAGGGAAG CAGAAGTCAC TGTGTAGGGA TAACCAAGGG    711

AGGGGAGGGG GGAAGGGAGG GGGAAAGGAA GAGTAGGAGA GGCCCAAACC CAAACCATAT    771

CTGGGGGGGC GTGGTTCTCT ACTGCCAAAC GCCCATCCTT GGAAGAAAGT TGTTGGCTAC    831

TATGCTGATG CTTCCTTGAG GCCACCAGAG AGTCCTCCTC TAGCCACCAA ATATGGCCCC    891

ATCTATCCTC AATTACCGAC ACTTGGGGCC TCACCAGCTG CCATTCCACT GGCGCCACTC    951

TTGAGGGTGA CTGCTGGGTC ATACACTGAG GTCTTGCAAA CCCATTCGTG TA           1003
```

FIG.2A

```
ACACACCCCA GCACATCAGC CCAGGAAACT TATAACCTCG GGAGTCAGGT CCCTCCCCTC    60

ACTGTGGTTG CAAATCTCCT GAAGAGAGGA CCAGACCAGC AGCCTGCTCT ACCACCCAGG   120

GCATCTGCCT CTCTCACTGG ATACTCCAGA ATTCTCTACT CAGAAGTCAC CAAAAAGCCA   180

AG ATG TTG GTG CTA CTG GCT GGT CTC TTT GTG GTC CAC ATT GCC ACT      227
   Met Leu Val Leu Leu Ala Gly Leu Phe Val Val His Ile Ala Thr
    1               5                  10                  15

GCC ATT ATG CTG TTT GTC TCC ACC ATT GCC AAC GTC TGG ATG GTT GCA     275
Ala Ile Met Leu Phe Val Ser Thr Ile Ala Asn Val Trp Met Val Ala
                 20                  25                  30

GAT TAC GCA AAT GCA TCT GTA GGG CTT TGG AAG AAC TGC ACT GGT GGT     323
Asp Tyr Ala Asn Ala Ser Val Gly Leu Trp Lys Asn Cys Thr Gly Gly
             35                  40                  45

AAC TGC GAC GGC TCC CTG TCC TAC GGC AAT GAA GAT GCT ATC AAG GCA     371
Asn Cys Asp Gly Ser Leu Ser Tyr Gly Asn Glu Asp Ala Ile Lys Ala
         50                  55                  60

GTG CAA GCC TTC ATG ATC CTC TCC ATC ATC TTC TCC ATC ATC TCC CTC     419
Val Gln Ala Phe Met Ile Leu Ser Ile Ile Phe Ser Ile Ile Ser Leu
     65                  70                  75

GTG GTC TTC GTG TTC CAG CTC TTC ACT ATG GAG AAG GGA AAC CGG TTC     467
Val Val Phe Val Phe Gln Leu Phe Thr Met Glu Lys Gly Asn Arg Phe
 80                  85                  90                  95
```

FIG.2B

```
TTC CTC TCG GGG TCC ACC ATG CTG GTG TGC TGG CTG TGT ATC CTG GTT       515
Phe Leu Ser Gly Ser Thr Met Leu Val Cys Trp Leu Cys Ile Leu Val
                100                 105                 110

GGA GTG TCA ATC TAC ACT CAT CAT TAC GCC CAC AGC GAA GGG AAC TTC       563
Gly Val Ser Ile Tyr Thr His His Tyr Ala His Ser Glu Gly Asn Phe
            115                 120                 125

AAC TCC AGC AGC CAC CAA GGC TAT TGT TTC ATC CTG ACC TGG ATC TGC       611
Asn Ser Ser Ser His Gln Gly Tyr Cys Phe Ile Leu Thr Trp Ile Cys
        130                 135                 140

TTC TGT TTC AGC TTC ATC ATC GGC ATA CTC TAT ATG GTC CTG AGG AAG       659
Phe Cys Phe Ser Phe Ile Ile Gly Ile Leu Tyr Met Val Leu Arg Lys
    145                 150                 155

AAA TAAGCCGAAT ACGCTCATGG GCGTCTGGGG GCGGGGTGGG CTGGGTAGGA           712
Lys
160

GGAAGCAACC TAACCTGGGA GGGAAGCAGG AGTCACTGTG TAGGAATAAC AGAGAGGGGA    772

GGGGGGTGGG GAGAGGGAAG GAAGAGGGGG AGAGGCCCAA ACCCAAACCA TATCTGGGGC    832

GGTGGGATTC TCTACTGCCA AGCACCCATC CTTGGAAGAA AGTTGTTGGC TGATATGCTG    892

ATGCTTCCTT GACGTCACCA GAGAGTCCTC CTCTAGCCAC CAAATATGGC CCCGTCCATC    952

CTCAATTACA TACACTCGGG GCCTCCCCAG CTGCCATACC ACTGGCGCCA CTCTTGAGGG   1012
```

FIG.2C

```
TGGCTGCTGG GTCACACACT GAGGTCTTCC ACATCCCATA TCATCAAGTT CTGATGGTGG    1072
TTCAGGTCTT AGCAAGAGCA GATATTGCTC GATGCTGAGG CTAAGTCTGG AAGCCACTTT    1132
GTCCTTGTGA CCTAAAACCA AACATCAAAT CCAGATCCCA TGTGCCTGTA GTGGGAGCTT    1192
TGGCCAGGAA GCCAATGTGC ATATTTGGTG GCCTTTCTAA CAAAAGTATA GGATGATGAG    1252
AGATGGTTTG TAAGTTCAAA GCTGATGGAA TTGGTTTAGC CAAGAAATGG AAGTTTCTAC    1312
CCCAGAGGAT CCTTGGAGAC AGGTGGGGAC AGGCAGTGCT CCTCAGTCAC GTGTCACCGA    1372
GCTGTCCCTC ATGGAGGCCT CCTGTTGTGA ACTCTGCTAG ACTCTCACTT ACAGCCAAGG    1432
CAGCTTTTCT GGAGTTTTTC TTAGATTCTC TAGAGCCAAA GATGATAATG CCTCACAAAA    1492
CATAGGGTCA AAGCATATGC CCACCGCAGT GCTATAGTAA GTTTGTGGGT TTTTAGGATT    1552
CCCCCAAAGC ACTCAATGTA TCTTGTATAT GTAACAGGGG AGAAATGCAT GTGTTCCTTT    1612
GACATACAAT TCTGAACTAG GAATATTTGA GGAAGTCCAA TGATGACCAA CAACACTGGG    1672
```

FIG.2D

```
GACCAGAATA TAACATCTAA ATGCAGTAGT CACTGTTGCT TTGACCTGGG CTGGAGTGGT   1732
CTCCTCTCAA CAGCTTTCAT CACACTATTT TCCAGCTAAA GATGGCAAAG CTGTAAGCCA   1792
ATTAACATAT ACACCAACCT AAACTAAAGA ACCAGTCCTG AGGGTGTGAG AAAGGTGCTA   1852
TCTGGTTATG GATTATTAAG CAAACCATAT TTCATTTATG TTGAGAAGAG AATGCCTGCC   1912
CTCAGGGAAA AAAAAATGTA ATTGTGTGAG ATGAATAAAG TCCTGGTGAT AGGCAGACAG   1972
TTTCTTTTTT AAAACAGGAG AAACTCTTAG GGCATCCAGA CAGATGGTAG CTAAATTGTT   2032
GGGGCTGCAG GGGTATTCCT GTATAAGACT TAGAGGTAGT ATGATATCTC AGATTTCTGC   2092
CTTAAAGGGC TTTCTTTTTA GAAATAGTTT CTTTTATTGC CCTTAGAAGA TCACCCCCAG   2152
GAAGAGTATG AGCTATCTTT TCTACATTTC TTTTCCTAGG AATATTCTTA TCCATTTCTT   2212
ATATACATTT CTTTTGGGAG GGAGTTTTTA TGCTATAGTT GCTGGTATTT ATGTAAAGGG   2272
ACCATTACTA AGTGTATTTC TCTAGCATAT TATGTTTAAG GGACGTGTGT A            2323
```

FIG.3A

```
AGCCAAC ATG TTG GTA TTG CTG GCT GGT ATC TTT GTG GTC CAC ATC GCT      49
        Met Leu Val Leu Leu Ala Gly Ile Phe Val Val His Ile Ala
         1               5                  10

ACT GTT ATT ATG CTA TTT GTT AGC ACC ATT GCC AAT GTC TGG TTG GTT      97
Thr Val Ile Met Leu Phe Val Ser Thr Ile Ala Asn Val Trp Leu Val
 15                  20                  25                  30

TCC AAT ACG GTA GAT GCA TCA GTA GGT CTT TGG AAA AAC TGT ACC AAC     145
Ser Asn Thr Val Asp Ala Ser Val Gly Leu Trp Lys Asn Cys Thr Asn
                 35                  40                  45

ATT AGC TGC AGT GAC AGC CTG TCA TAT GCC AGT GAA GAT GCC CTC AAG     193
Ile Ser Cys Ser Asp Ser Leu Ser Tyr Ala Ser Glu Asp Ala Leu Lys
             50                  55                  60

ACA GTG CAG GCC TTC ATG ATT CTC TCT ATC ATC TTC TGT GTC ATT GCC     241
Thr Val Gln Ala Phe Met Ile Leu Ser Ile Ile Phe Cys Val Ile Ala
         65                  70                  75

CTC CTG GTC TTC GTG TTC CAG CTC TTC ACC ATG GAG AAG GGA AAC CGG     289
Leu Leu Val Phe Val Phe Gln Leu Phe Thr Met Glu Lys Gly Asn Arg
     80                  85                  90

TTC TTC CTC TCA GGG GCC ACC ACA CTG GTG TGC TGG CTG TGC ATT CTT     337
Phe Phe Leu Ser Gly Ala Thr Thr Leu Val Cys Trp Leu Cys Ile Leu
 95                 100                 105                 110

GTG GGG GTG TCC ATC TAC ACT AGT CAT TAT GCG AAT CGT GAT GGA ACG     385
Val Gly Val Ser Ile Tyr Thr Ser His Tyr Ala Asn Arg Asp Gly Thr
                115                 120                 125
```

FIG.3B

```
CAG TAT CAC CAC GGC TAT TCC TAC ATC CTG GGC TGG ATC TGC TTC TGC      433
Gln Tyr His His Gly Tyr Ser Tyr Ile Leu Gly Trp Ile Cys Phe Cys
            130                 135                 140

TTC AGC TTC ATC ATC GGC GTT CTC TAT CTG GTC CTG AGA AAG AAA          478
Phe Ser Phe Ile Ile Gly Val Leu Tyr Leu Val Leu Arg Lys Lys
        145                 150                 155

TAAGGCCGGA CGAGTTCATG GGGATCTGGG GGGTGGGGAG GAGGAAGCCG TTGAATCTGG    538

GAGGGAAGTG GAGGTTGCTG TACAGGAAAA ACCGAGATAG GGGAGGGGGG AGGGGGAAGC    598

AAAGGGGGGA GGTCAAATCC CAAACCATTA CTGAGGGGAT TCTCTACTGC CAAGCCCCTG    658

CCCTGGGGAG AAAGTAGTTG GCTAGTACTT TGATGCTCCC TTGATGGGGT CCAGAGAGCC    718

TCCCTGCAGC CACCAGACTT GGCCTCCAGC TGTTCTTAGT GACACACACT GTCTGGGGCC    778

CCATCAGCTG CCACAACACC AGCCCCACTT CTGGGTCATG CACTGAGGTC CACAGACCTA    838

CTGCACTGAG TTAAAATAGC GGTACAAGTT CTGGCAAGAG CAGATACTGT CTTTGTGCTG    898

AATACGCTAA GCCTGGAAGC CATCCTGCCC TTCTGACCCA AAGCAAAACA TCACATTCCA    958

GTCTGAAGTG CCTACTGGGG GGCTTTGGCC TGTGAGCCAT TGTCCCTCTT TGGAACAGAT   1018

ATTTAGCTCT GTGGAATTCA GTGACAAAAT GGGAGGAGGA AAGAGAGTTT GTAAGGTCAT   1078
```

FIG.3C

```
GCTGGTGGGT TAGCTAAACC AAGAAGGAGA CCTTTTCACA ATGGAAAACC TGGGGGATGG 1138
TCAGAGCCCA GTCGAGACCT CACACACGGC TGTCCCTCAT GGAGACCTCA TGCCATGGTC 1198
TTTGCTAGGC CTCTTGCTGA AAGCCAAGGC AGCTCTTCTG GAGTTTCTCT AAAGTCACTA 1258
GTGAACAATT CGGTGGTAAA AGTACCACAC AAACTATGGG ATCCAAGGGG CAGTCTTGCA 1318
ACAGTGCCAT GTTAGGGTTA TGTTTTTAGG ATTCCCCTCA ATGCAGTCAG TGTTTCTTTT 1378
AAGTATACAA CAGGAGAGAG ATGGACATGG CTCATTGTAG CACAATCCTA TTACTCTTCC 1438
TCTAACATTT TTGAGGAAGT TTTGTCTAAT TATCAATATT GAGGATCAGG GCTCCTAGGC 1498
TCAGTGGTAG CTCTGGCTTA GACACCACCT GGAGTGATCA CCTCTTGGGG ACCCTGCCTA 1558
TCCCACTTCA CAGGTGAGGC ACCGGAATTC TGGAAGCTGA TTAAAACACA CATAAACCAA 1618
AACCAAACAA CAGGCCCTTG GGTGAAAGGT GCTATATAAT TGTGAAGTAT TAAGCCTACC 1678
GTATTTCAGC CATGATAAGA ACAGAGTGCC TGCATTCCCA GGAAAAT 1725
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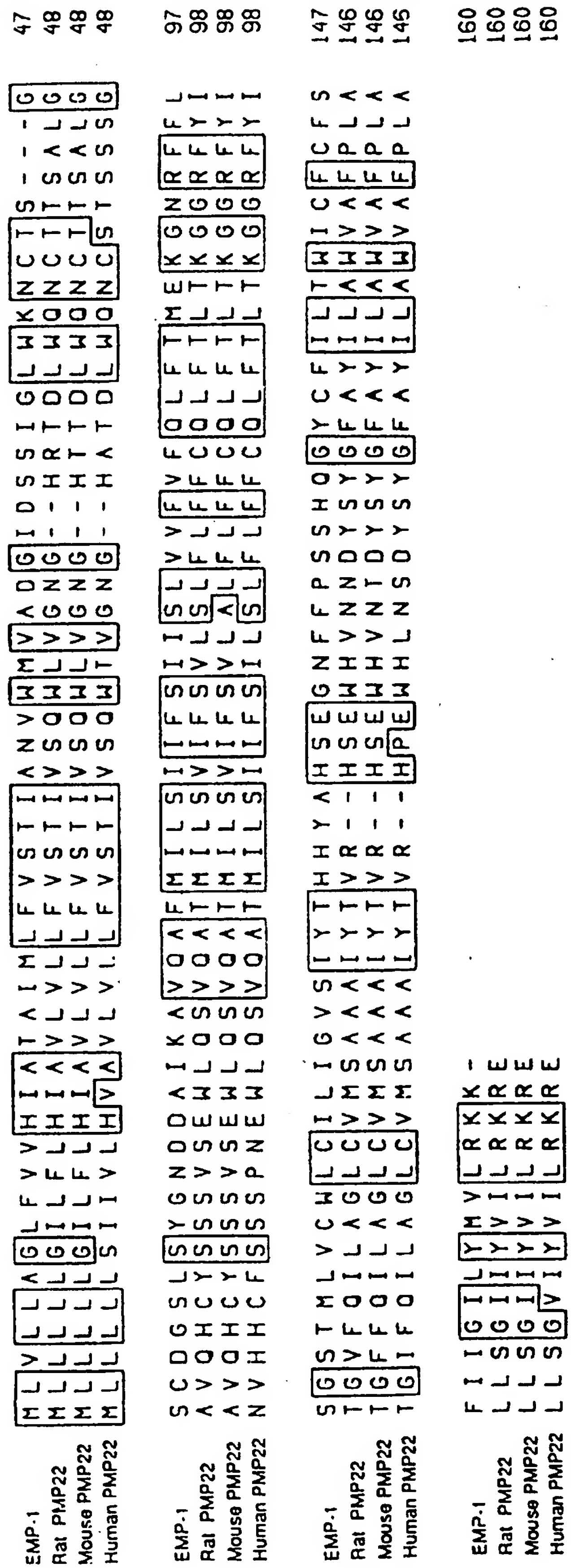


FIG.4A

FIG.4B

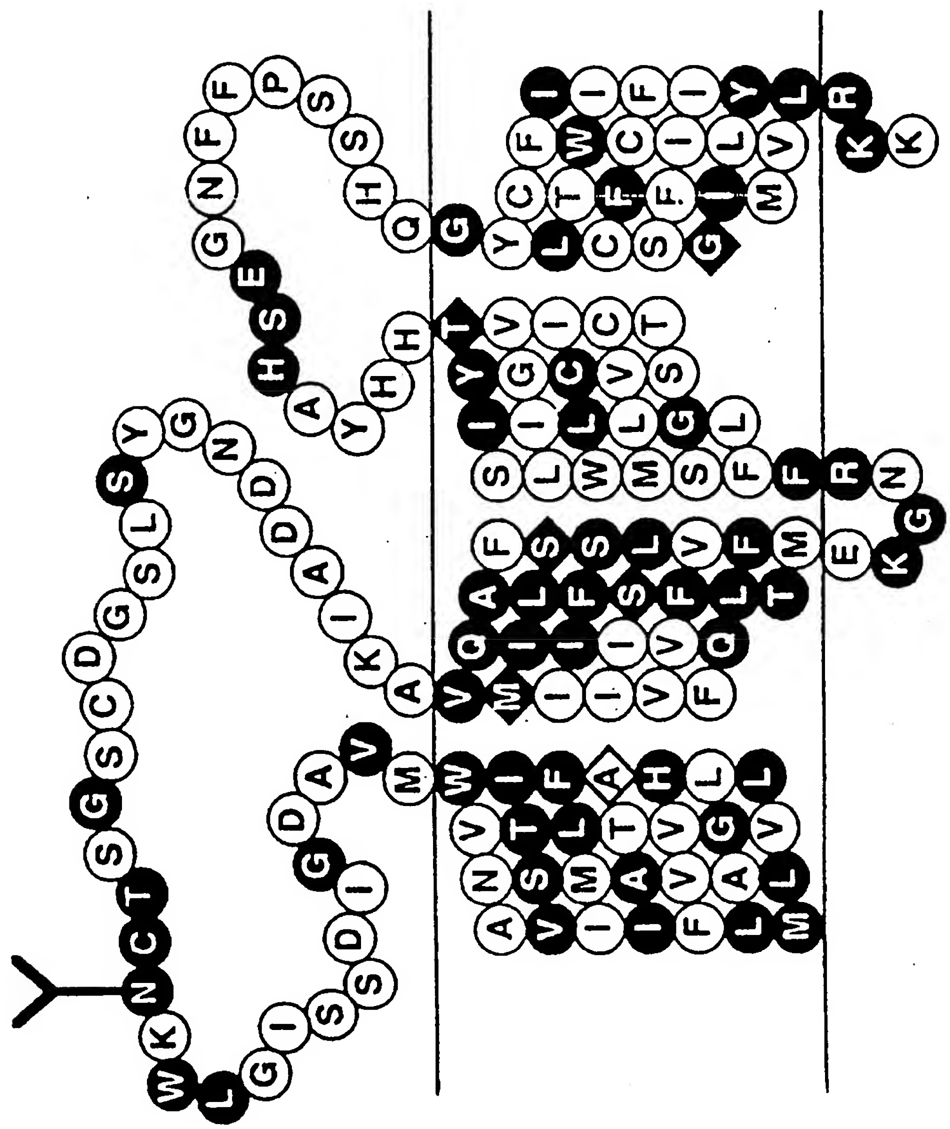
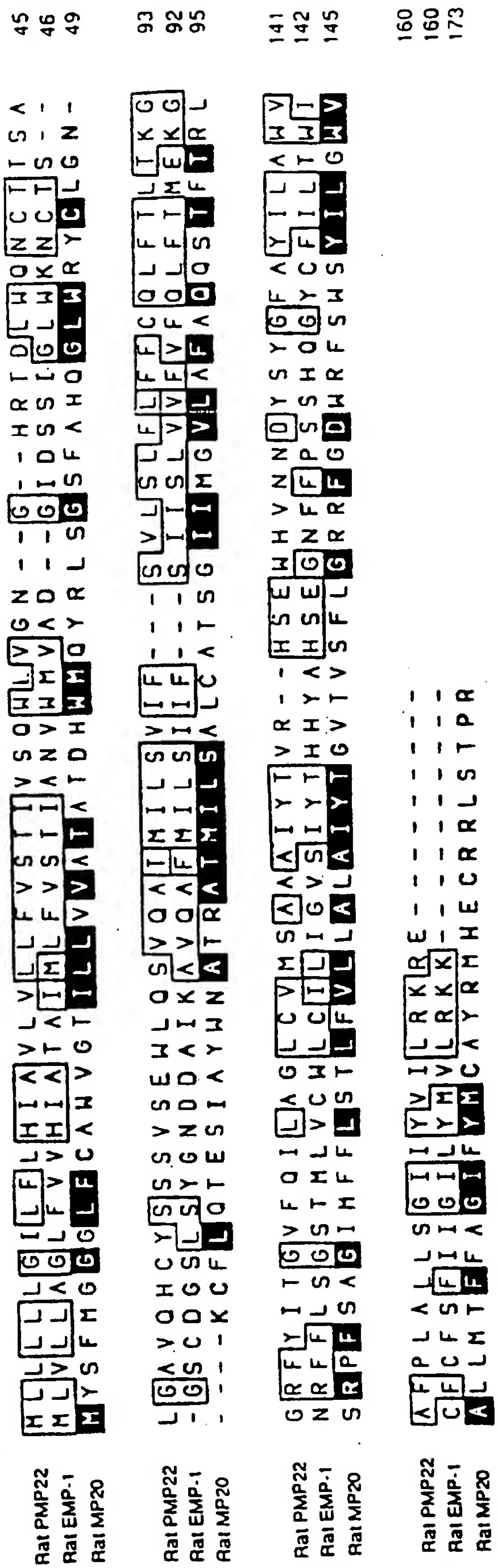


FIG.4C


Rat PMP22 M L L L L L G I L F L H I A V L V L L F V S T I V S Q W L V G N - - G - - H R T D L W Q N C T I S A 45
Rat EMP-1 M L V L L A G L F V V H I A T A I M L F V S T I A N V W M V A D - - G I D S S L G L W K N C T S - - 46
Rat MP20 M Y S F M G G G L F C A W V G T I L L V V A T A T D H W M Q Y R L S G S F A H Q G L W R Y C L G N - 49
Rat PMP22 L G A V Q H C Y S S S V S E W L Q S V Q A T M I L S V I F - - - S V L S L F L F F C Q L F T L T K G 93
Rat EMP-1 - G S C D G S L S Y G N D D A I K A V Q A F M I L S I I F - - - S I I S L V V F V F Q L F T M E K G 92
Rat MP20 - - - - K C F L Q T E S I A Y W N A T R A T M I L S A L C A T S G I I M G V L A F A Q Q S T F T R L 95
Rat PMP22 G R F Y I T G V F Q I L A G L C V M S A A A I Y T V R - - H S E W H V N N D Y S Y G F A Y I L A W V 141
Rat EMP-1 N R F F L S G S T M L V C W L C I L I G V S I Y T H H Y A H S E G N F F P S S H Q G Y C F I L T W I 142
Rat MP20 S R P F S A G I M F F L S T L F V L L A L A I Y T G V T V S F L G R R F G D W R F S W S Y I L G W V 145
Rat PMP22 A F P L A L L S G I I Y V I L R K R E - - - - - - - - - - 160
Rat EMP-1 C F C F S F I I G I L Y M V L R K K - - - - - - - - - - - 160
Rat MP20 A L L M T F F A G I F Y M C A Y R M H E C R R L S T P R 173

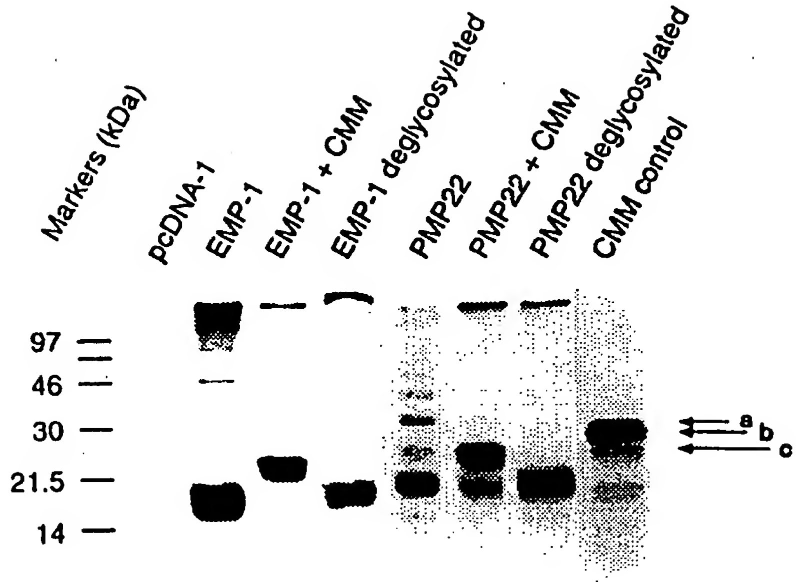

Markers (kDa)
pcDNA-1
EMP-1
EMP-1 + CMM
EMP-1 deglycosylated
PMP22
PMP22 + CMM
PMP22 deglycosylated
CMM control
97
46
30
21.5
14
a
b
c

FIG.5

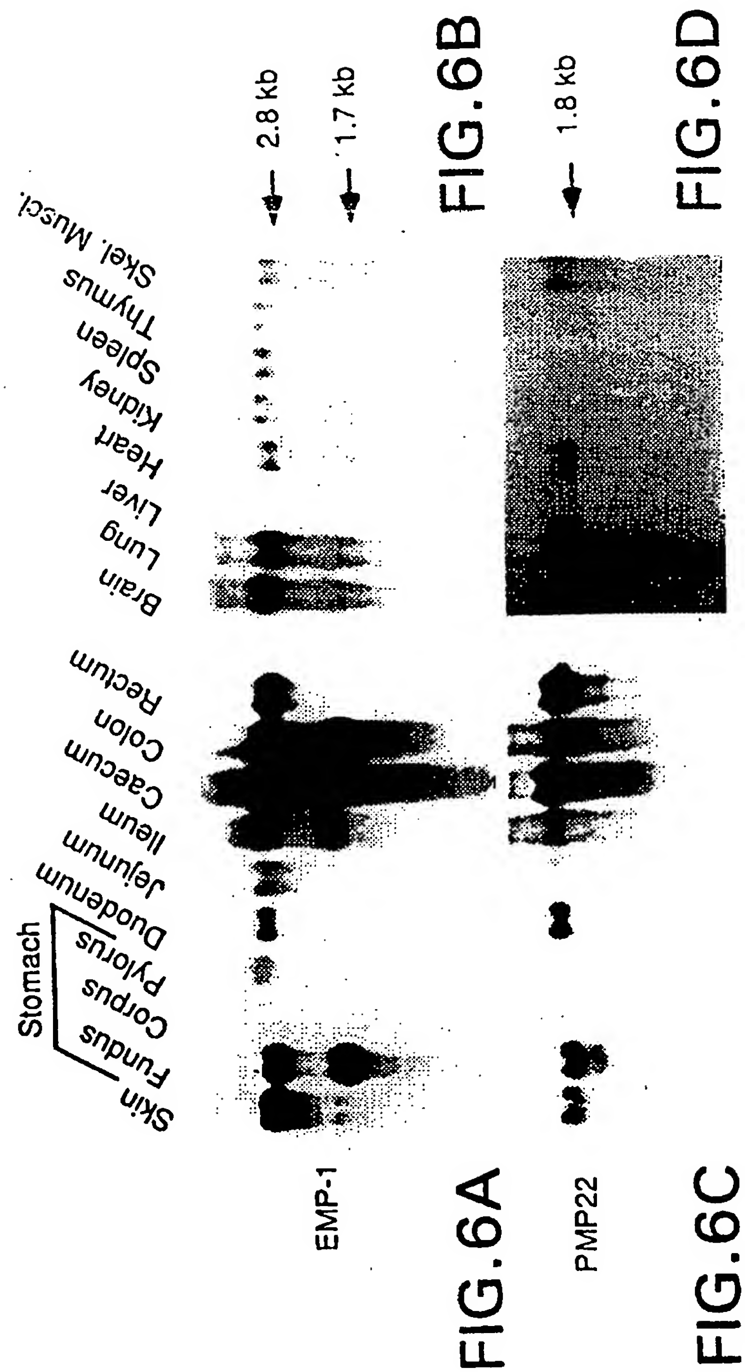


FIG.6A

FIG.6B

FIG.6C

FIG.6D

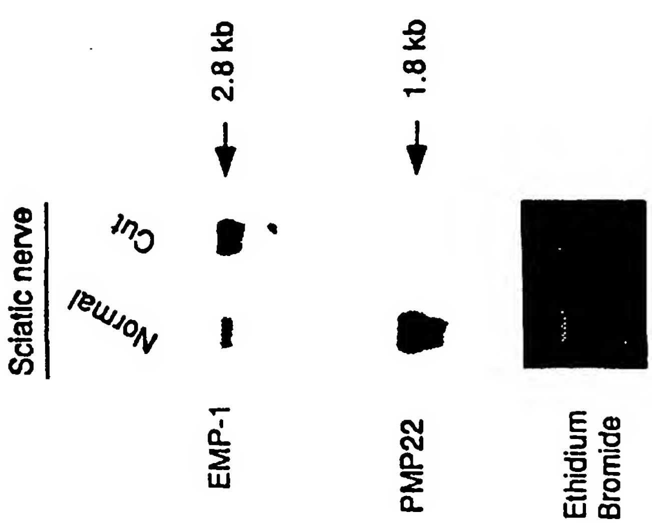


FIG.7A

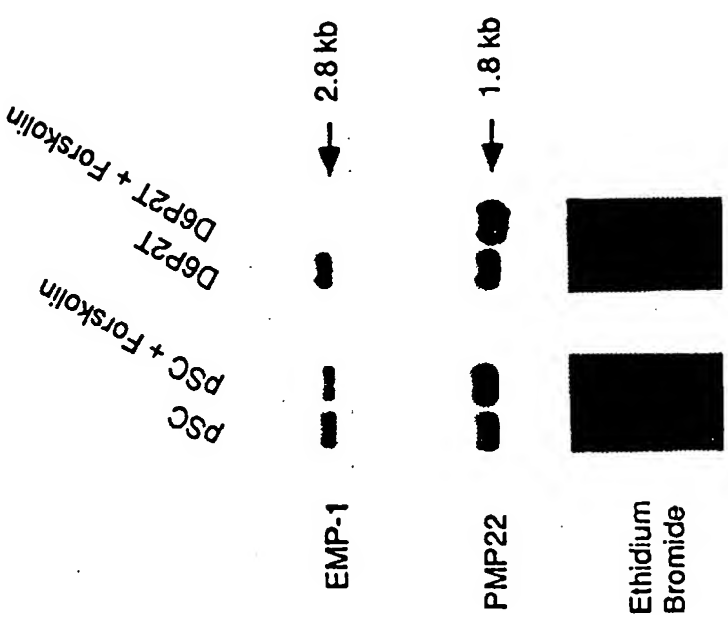


FIG.7B

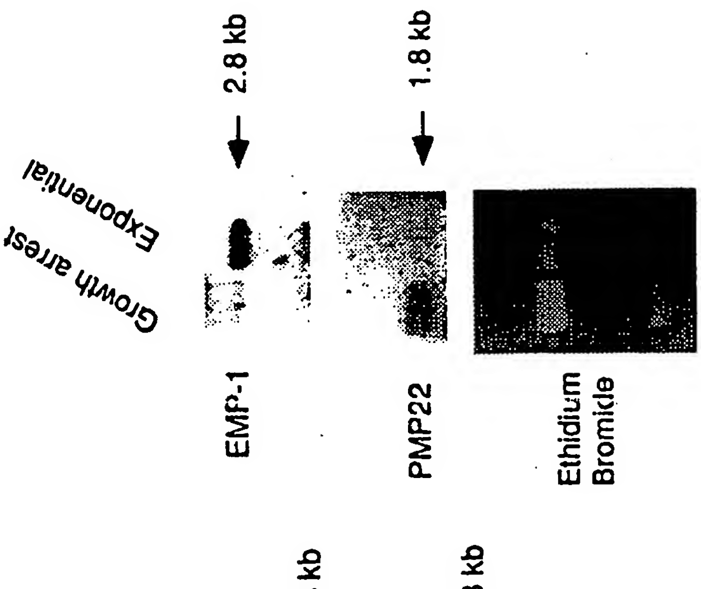


FIG.7C

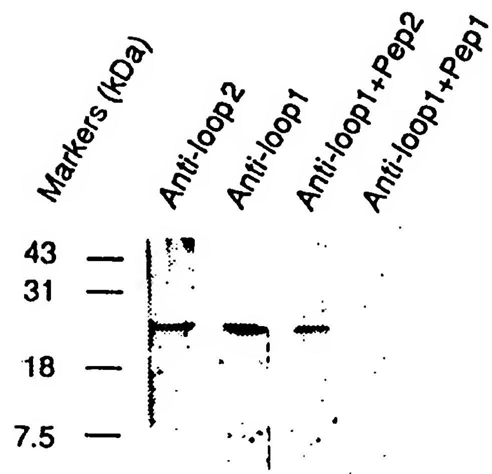

Markers (kDa)
Anti-loop2
Anti-loop1
Anti-loop1+Pep2
Anti-loop1+Pep1
43
31
18
7.5

FIG.8A


Markers (kDa)
Stomach
Lung
Liver
Fundus
Corpus
Pylorus
Duodenum
Jejunum
Ileum
Caecum
Colon
43
31
18
7.5

FIG.8B

FIG.9

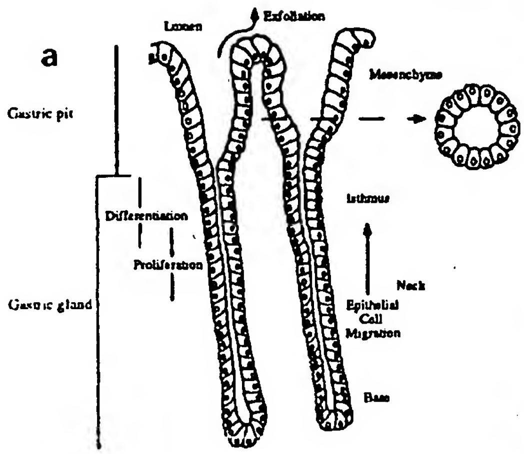


FIG.10A

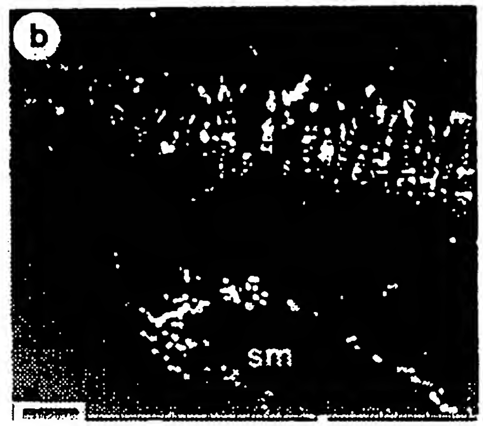


FIG.10B

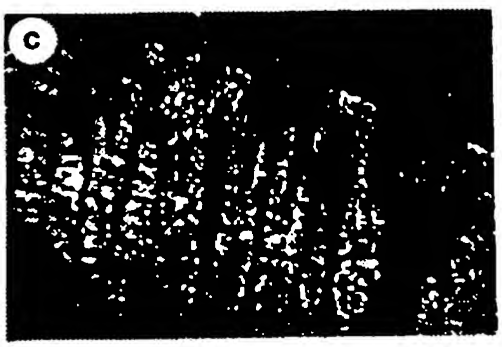


FIG.10C

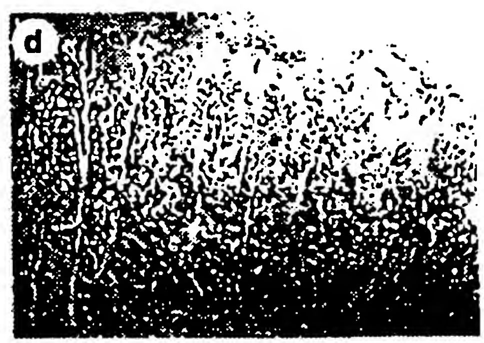


FIG.10D

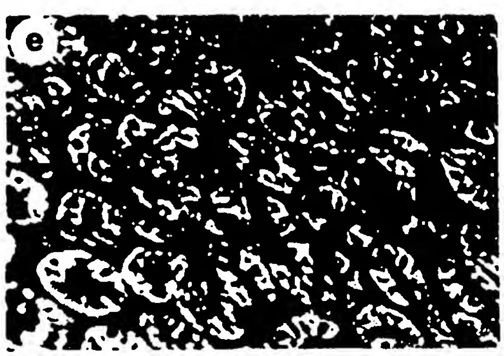


FIG.10E

FIG.10F

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PC ı/US 96/18852

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705 C07K16/30 A61K48/00 C12Q1/68 G01N33/50 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	J BIOL CHEM, DEC 1 1995, 270 (48) P28824-33, UNITED STATES, XP000645327 TAYLOR V ET AL: "Epithelial membrane protein-1, peripheral myelin protein 22, and lens membrane protein 20 define a novel gene family." see figure 1 ---	1-3,6-22
P,X	GENOMICS, SEP 15 1996, 36 (3) P379-87, UNITED STATES, XP000645625 LOBSIGER CS ET AL: "Identification and characterization of a cDNA and the structural gene encoding the mouse epithelial membrane protein-1." see figure 1 ---	1,2,4, 6-22
	-/--	

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 1997	25.03.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Espen, J

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/US 96/18852

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF IMMUNOLOGY, vol. 157, no. 1, 1 July 1996, pages 72-80, XP000616417 RUEGG C L ET AL: "B4B, A NOVEL GROWTH-ARREST GENE, IS EXPRESSED BY A SUBSET OF PROGENITOR/PRE-B LYMPHOCYTES NEGATIVE FOR CYTOPLASMIC MU-CHAIN" see figure 2 ---	1,2,5-22
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 48, 1 December 1995, pages 28910-28916, XP000616413 MARVIN K W ET AL: "IDENTIFICATION AND CHARACTERIZATION OF A NOVEL SQUAMOUS CELL-ASSOCIATED GENE RELATED TO PMP22" see figure 2 ---	11-13
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 88, 1991, WASHINGTON US, pages 7195-7199, XP002027824 WELCHER AA ET AL.: "A myelin protein is encoded by the homologue of a growth arrest-specific gene" cited in the application see the whole document -----	11-13

1

International application No.

INTERNATIONAL SEARCH REPORT

PCT/US 96/18852

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 16-18
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.